# LABORATORY AIDS
## IN
# ENDOCRINE DIAGNOSIS

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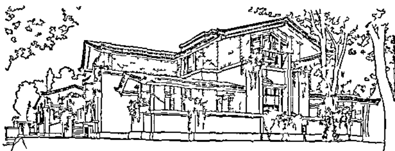
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## DEDICATION

*To* DOCTOR HANS LISSER—*stimulating teacher preceptor and colleague through years of training in that exacting art and science—the practice of clinical endocrinology*

## CONTENTS

# LABORATORY AIDS IN ENDOCRINE DIAGNOSIS

*Chapter I*

# INTRODUCTION

IN CLINICAL practice laboratory tests frequently aid in the diagnosis and treatment of endocrinopathies. Many of the tests to be described are widely used—at times perhaps overused and given undue emphasis in the management of the patient. Therefore the word "Aids" has been purposely incorporated in the title in order to relegate laboratory procedures to their proper rank in relation to a thorough clinical examination.

Laboratory findings are for the most part only aids or adjuncts not only in diagnosis but also in following the effects of treatment in endocrine disease. They do not replace or transcend a proper and complete anamnesis and physical examination of the patient. Furthermore the fallibility of many of the tests is well known to anyone who has worked for any length of time in the laboratory. Many of the procedures are complicated and some of the steps may be uncertain even though the final results are expressed in exact figures. This persuasive preciseness may lead to a false sense of security so that at times serious errors can occur unless laboratory findings are correlated with clinical impressions. If the laboratory results indicate conclusions at variance with clinical facts it is prudent to place more credence in the clinical observations and either repeat the tests (perhaps in another laboratory) or if necessary repeat or re-evaluate entire experiments. At times it is advantageous with some of the more complicated tests to confine them to one particular laboratory or even to repose confidence in one specially trained technician in interpreting results for clinical use.

On the other hand lest the de emphasis be carried too far laboratory results occasionally can be most helpful and even definitive in diagnosis e g the level of FSH excretion in the urine is of critical importance in distinguishing between pituitary infantilism and ovarian aplasia (being elevated in the latter) The laboratory may also be helpful in checking results of treatment—as in following the level of plasma cholesterol or the basal metabolic rate during treatment of hypothyroidism with desiccated thyroid substance Also laboratory studies have increased our knowledge about physiological changes in several of the endocrine diseases and thus embellish and clarify the clinician s understanding of the patient and his disease in a manner beneficial to both physician and patient

In the following pages the various tests will be listed noting the principal abnormalities found in the presently acknowledged endocrinopathies In the final chapter (XII) this data is recapitulated in the form of a disease index listing the various endocrine diseases and collecting under these headings the appropriate laboratory findings This opposite approach offers the reader a cross reference to the material presented and provides a substitute for the customary index

The tests listed are those which have been proved to have some clinical value plus a few that seem particularly promising at this time Laboratory procedures which still are highly experimental or are confined to research projects have been purposely omitted In some instances more than one method is in use for the same test The method given will be the one currently used in the laboratories in or connected with the University of California Hospital or Medical School

In compiling a review of this type one must rely heavily on the generous help of friendly colleagues Sin

cere thanks are due to the following individuals who have been most kind in giving advice reading sections and cooperating in general Dr James Hopper Dr Morris Dailey Dr Peter Forsham Dr Felix Kolb Dr Maurice Sokolow and Dr Hans Lisser of the Department of Medicine Dr Ernest Page Dr Ralph Benson and Dr M James Whitelaw of the Department of Obstetrics and Gynecology Dr Donald Smith and Dr Frank Hinman Jr of the Department of Urology Dr A Justin Williams and Dr Earl R Miller of the Department of Roentgenology and Dr Norman Freeman of the Department of Surgery

Others have been most helpful in allowing the use of photographs and charts for some of the illustrations Each will be acknowledged at its point of appearance in the text

R F E

*Chapter II*

# INFORMATION CONCERNING ENDOCRINE DISEASES FROM THE USUAL ROUTINE LABORATORY TESTS

EVEN the most *common* tests show *important* abnormalities in some endocrine diseases

## BLOOD COUNTS

A complete count including estimation of amount of hemoglobin numbers of red cells and white cells differential count of the white corpuscles and examination of the stained smear is a routine procedure with most physicians and certainly with most hospital entries

In patients with *Cushing s syndrome*, *polycythemia* with increase in hemoglobin (90 to 120%) and number of red blood corpuscles (5 to 6 500 000) is a characteristic finding Polycythemia is also seen in *diabetic acidosis* and in *parathyroid tetany*

Moderate *secondary anemia* is found frequently in *myxedema* In this condition the hemoglobin is low in relation to the red cell count so that the color index is less than one (color index can be estimated by doubling the first two numbers of the erythrocyte count and dividing this into the hemoglobin percentage) Occasional instances have been reported of the association of a *primary anemia* with *myxedema* In these the hemoglobin is high in relation to the red cell count so that the color index is greater than one

*Secondary anemia* is also found with some frequency in *Simmonds disease Addison s disease severe hyperthyroidism* and late in the course of *hyperparathyroidism*

*Eosinophilia*—an increase in percentage of eosinophiles in the differential count of white blood corpuscles to above the usual normal level of 1 to 3% is found with some frequency in many endocrine diseases It has been reported in more than average incidence in both *Simmonds disease* and *acromegaly* which are physiological opposites as regards pituitary function It is also seen occasionally in *Addison s disease severe hyperthyroidism* and late *hyperparathyroidism* Count of the circulating eosinophiles as described in Chapter III is now used as a test of adrenal cortical function

A *relative lymphocytosis* so called when the percentage of lymphocytes in the differential count is above the usual average of 25 to 30% with a normal total white cell count is another frequent finding in many endocrine diseases It has been reported in *acromegaly pituitary infantilism hyperthyroidism myxedema parathyroid tetany Addison s disease* and *hypogonadism* As was noted in discussing eosinophilia many of these conditions are physiological opposites It is of interest to recall that before basal metabolic rate determinations were available the finding of a *leucopenia* with a relative *lymphocytosis* was considered an important diagnostic sign in *hyperthyroidism*

An increase in the total *white blood cell count* is seen in *diabetic acidosis* leucocytosis to a level as high as 75 000 white cells per cu mm is not uncommon

## COMPLETE URINALYSIS

This is another commonly performed test Usually the color turbidity specific gravity and degree of acidity or alkalinity are noted Tests are performed for albumin and glucose and frequently for acetone and the sediment is examined microscopically In some laboratories a Sulkowitch test for rough estimation of the amount of calcium

in the urine is done routinely as is discussed in Chapter IV

The finding of *glucose* in the urine with associated *high specific gravity* is important in the diagnosis of *diabetes mellitus* It must be remembered that diabetes mellitus is an occasional complication of *acromegaly* and it has been variously estimated that 25 to 40% of acromegalics become diabetic In *Cushing's syndrome,* most of the untreated patients eventually develop diabetes Glycosuria is also seen occasionally in *gigantism hyperthyroidism* and with *adrenal cortical tumors* (especially the Achard Thier Syndrome— Diabetes of Bearded Women )

*Diabetes insipidus* may be suspected from urinary findings alone The 24 hr volume (normally one to two liters) will be increased to over three liters and up to a reported maximum of 56 liters Specific gravity is low—usually 1 001 or 1 002—and the urine looks pale watery and tastes insipid Neither albumin nor sugar are found to be present The condition is a disease entity but may occasionally be a complication of tumors or other lesions involving the pituitary or hypothalamus Such tumors may also cause various endocrine diseases including *gigantism, acromegaly pituitary infantilism Froehlich's syndrome Simmonds disease* or *precocious puberty* and thus diabetes insipidus may be associated with all of these clinical syndromes

Evidence of *kidney damage* with albumin casts and fixed specific gravity is seen in the late hypertensive phase of *Cushing's syndrome* and occasionally in *diabetes mellitus* when the Kimmelstiel Wilson syndrome of intercapillary glomerular sclerosis has appeared Albumin may also be found during hypertensive attacks with *pheochromocytoma*

*Hyperparathyroidism* is characterized by increased

urinary excretion of calcium so that in untreated cases lithiasis nearly always develops and eventually is further complicated by infection This results in the appearance of red cells white cells albumin casts and particularly calcium casts in the urine

Untreated *myxedema* frequently is complicated by *atony of the bladder* This results in incomplete emptying stasis and residual urine which usually becomes infected so that pus cells and albumin are frequently found

*Acetone bodies* as acetone or diacetic acid are found in *diabetic acidosis* or may also be present if the patient is on a semi starvation diet

### SEROLOGICAL TESTS FOR SYPHILIS

These are performed with enough frequency to be considered a routine test They may be of some importance in *diabetes insipidus* which can be caused by infection or gumma in the hypothalamus or pituitary region Also some instances of *Simmonds disease* have been reported to result from a gumma replacing and destroying the anterior lobe of the pituitary gland

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The finding of *glucose* in the urine with associated *high specific gravity* is important in the diagnosis of *diabetes mellitus*. It must be remembered that diabetes mellitus is an occasional complication of *acromegaly* and it has been variously estimated that 25 to 40% of acromegalics become diabetic. In *Cushing's syndrome* most of the untreated patients eventually develop diabetes. Glycosuria is also seen occasionally in *gigantism*, *hyperthyroidism* and with *adrenal cortical tumors* (especially the Achard Thier Syndrome— Diabetes of Bearded Women.)

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*Hyperparathyroidism* is characterized by increased

Stimulation of the adrenal cortical function in normal individuals will cause a fall of at least 50% in the number of circulating eosinophiles However in *Addison s disease* the count is high or high normal and does not drop after efforts to stimulate the adrenal cortex with adrenocortico tropic hormone (ACTH) or with epinephrine

## THE FOUR HOUR CORTICOTROPIN (ACTH) TEST

*The four hour corticotropin (ACTH) test of adrenal cortical function* is based on the above

Method An eosinophile count is made with the patient in a fasting state and then 25 units of corticotropin (ACTH) are administered intramuscularly Four hours later another blood sample is drawn and the eosinophiles again counted Breakfast may be eaten after the first count but lunch should be withheld until the second count is taken *Normally* the count should drop at least 50% from the stimulation of the adrenal cortex by the corticotropin If it does not adrenal cortical insufficiency is a possibility

Recently Bonner has suggested that the test may be more sensitive if done in the non fasting patient from one to five p m

## THE 48 HOUR CORTICOTROPIN (ACTH) TEST

This variation is used when the four hour test does not result in clear differentiation between primary and sec ondary adrenal cortical disease

Method A fasting count of circulating eosinophiles is done and then 10 units of corticotropin (ACTH) are ad ministered intramuscularly at 10 a m on the first day The dose of 10 units is repeated every six hours for 48 hours (eight injections)—giving the last injection at 4 a m of the third day Another eosinophile count is taken four hours

*Chapter III*

# SPECIAL TESTS OF BLOOD

## EOSINOPHILE COUNT

THE DIRECT count of circulating eosinophiles has been found to be of value in estimation of adrenal cortical function as reported by Thorn Forsham and co-workers[1]

Method Five cc of venous blood is collected with 0 5 cc of dried balanced oxalate solution in the tube A standard white cell pipette is used drawing the blood to the 0 5 mark This is diluted with a special stain to the 11 mark The stain is prepared by mixing 5 cc of 2% aqueous eosin 5 cc of acetone and 90 cc of distilled water This should be kept in a refrigerator and filtered before use It should be prepared fresh every two weeks Originally a special counting chamber was used with a depth of 0 2 mm but it has been found that a standard counting chamber with a depth of 0 1 mm is satisfactory The pipette is shaken for only 30 seconds and the counting chamber filled immediately and allowed to stand for 3 minutes Most of the red blood cells and other white cells are hemolyzed while the eosinophiles are identified by the deeply stained red granules Four chambers of 16 sq mm are counted The total is divided by 16 and multiplied by 100 to give the circulating eosinophiles per cu mm Cleanliness is essential as the cells tend to cluster around foreign bodies and timing is important as they are unstable in the diluent

*The normal range* is 150 to 350 per cu mm Recently Ponner has suggested that an eosinophile estimation from the differential count on a smear is as satisfactory as the Chamber method if 800 cells are counted

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*later (at 8 a m ) before allowing breakfast* Normal individuals show a fall of over 50% while those with Addison s disease consistently fail to fall to 50% of the original level 17 Ketosteroid excretion can also be checked before and after the course of corticotropin (ACTH) and normally rises to and above the upper limits of normal excretion

Patients with *hypopituitarism* and *secondary adrenal cortical atrophy* show an eosinophile drop greater than the Addisonian and usually over 50%

It is of interest to note that the *lymphocytes* respond in the same manner as the eosinophiles in the above tests—but the eosinophile count is at present the more accepted

## BLOOD SUGAR DETERMINATIONS AND GLUCOSE TOLERANCE TESTS

These are occasionally of considerable importance in endocrine diseases The method of measuring the blood sugar is now in a state of flux The older is the classical *Folin Wu* method and is the one still used in most laboratories because of its proven dependability However it does *measure some reducing substances other than glucose so* the *Somogyi method* which measures only glucose is being set up in some laboratories and is the present one in use at the University of California Hospital Values with the Somogyi procedure are generally about 20 mg below those found with the older Folin Wu so a knowledge of which method is used may be important in the clinical interpretation Both methods follow

**Folin Wu method** Venous blood is drawn into an oxalate tube *A protein free filtrate* is prepared as follows One cc of blood is diluted with 7 cc of distilled water and mixed One cc of 10% sodium tungstate is then added and after mixing 1 cc of 2/3 N sulfuric acid is

added This results in a dark brown mixture which is filtered yielding a water clear filtrate The *sugar test* is dependent upon the reaction of the sugar with an alkaline copper solution—this results in a blue color the depth of which is in proportion to the amount of sugar Two cc of the clear filtrate is placed in a blood sugar test tube (graduated at 25 cc) and 2 cc. of two standard sugar solutions are placed in each of two other tubes The standard solutions contain respectively 1 mg of sugar per 10 cc and 2 mg of sugar per 10 cc thus the 2 cc. of each contain 0 2 mg and 0 4 mg of dextrose To each of the three tubes is added 2 cc of alkaline copper solution (prepared by dissolving 40 gm of anhydrous sodium carbonate in 400 cc of water adding 7 5 gm of tartaric acid and then 4 5 gm of crystallized copper sulfate made up to a volume of one liter) The three tubes are boiled in a water bath for 6 minutes and then cooled in cold water Two cc of molybdate phosphate solution is then added to each tube (This is prepared by mixing 35 gm of molybdic acid and 5 gm of sodium tungstate in 200 cc of 10% sodium hydroxide and 200 cc of water The mixture is boiled for 20 to 40 minutes to remove the ammonia cooled diluted to 350 cc and then mixed with 125 cc of concentrated (85%) phosphoric acid Final dilution to 500 cc is then performed)

A blue color forms and the volume is then made up to 25 cc with distilled water After mixing the unknown and the weaker standard are compared in the colorimeter The depth of the standard (in mm) is multiplied by 100 and divided by the reading of the unknown giving the sugar content in mg per 100 cc of blood If the stronger standard is used the depth of the standard is multiplied by 200

Somogyi method[3] (blood glucose only) Venous blood is drawn and placed in an oxalate tube In *preparation of the filtrate* 1 cc of the oxalated blood is mixed with 15 cc. of distilled water and 2 cc of barium hydroxide solution

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is adjusted to give 100% transmission through the blank An Evelyn 515 filter (green) is used which gives maximum transmission at 500 to 520 millimicrons

The blood glucose values are then read directly from a graph with curve made up from standard solutions [4]

Glucose tolerance tests are generally satisfactory using the classical method as follows

Fasting blood is drawn and urine collected and then the patient drinks 100 gm of glucose in water flavored with lemon Blood and urine specimens are taken at 30 minutes one two three and occasionally four five and six hours afterwards The longer tests are performed particularly if a hypoglycemic tendency is suspected as the hypoglycemic dip in the curve may not occur until the fourth fifth or sixth hour

Some variations have been suggested in the method of performing this test such as giving the glucose according to the weight of the patient (justified in children) giving glucose more than once or giving the glucose intravenously (occasionally justified if absorption from the gastrointestinal tract is impaired or abnormal) However the method given above is generally satisfactory

There has been some discussion concerning the necessity for special diet before performing the glucose tolerance test In general the preceding diet will not grossly affect the glucose tolerance curve if the carbohydrate intake has been between 100 gm and 300 gm daily Nearly all diets afford an intake of carbohydrate within these limits —the possible exception being in a patient with severe anorexia nervosa Such a patient should be fed a high carbohydrate diet several days before the test in order to obtain a true picture of his capacity to handle glucose

*The normal glucose tolerance curve* is as follows Fasting level between 90 and 120 mg % (Folin Wu method) with a rise to a peak of 130 to 160 mg % in one half to one hour followed by a fall to the fasting level within 2 hours

added (The latter is prepared by adding 24 gm $Ba(OH)_2$ to enough water to fill a 500 cc volumetric flask making 0.3 N $Ba(OH)_2$. This is adjusted with zinc sulfate solution (preparation given below) to a pink end point using phenolphthalein as an indicator. Further adjustment is made by adding water until 4.7 to 4.8 cc of barium hydroxide are required to titrate the zinc sulfate. The solution should be stored in a bottle protected from the $CO_2$ of the air by soda lime.)

The mixture turns brown and then 2 cc of zinc sulfate solution are added (prepared by dissolving 25 gm of $ZnSO_4 \cdot 7H_2O$ in distilled water and bringing to volume in a 500 cc volumetric flask). This is filtered and yields a clear filtrate. Glucose determination is accomplished by placing 1 cc of the filtrate in a sugar tube graduated at 25 cc. In a similar tube is placed 1 cc of distilled water. One cc of copper reagent is added to each. (The copper reagent is prepared by dissolving 28 gm of anhydrous disodium phosphate and 40 gm of Rochelle salt in 700 cc of water and then adding 100 cc of normal sodium hydroxide. Eighty cc of a 10% copper sulfate solution are added with stirring and then 180 gm of anhydrous sodium sulfate. This is diluted to a liter, allowed to stand for 1 to 2 days and filtered.)

The solutions in the tubes are mixed and then heated for 10 minutes in a boiling water bath. After cooling in cold water, 1 cc of arsenomolybdate color reagent is added to each tube, mixed and diluted to 25 cc with distilled water. They are then mixed and read in a photoelectric colorimeter. (The arsenomolybdate color reagent is prepared by dissolving 25 gm of ammonium molybdate in 450 cc of distilled water, adding 25 cc of concentrated $H_2SO_4$, mixing and adding 3 gm of $Na_2HAsO_4 \cdot 7H_2O$ dissolved in 25 cc of water. This is mixed, incubated at 37 C for 24 to 48 hours and then stored in a brown glass stoppered bottle.)

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Such a normal curve may be important in the diagnosis of *diabetes insipidus*

*A high glucose tolerance curve* (indicating low tolerance) is found classically in *diabetes mellitus* It is also seen when diabetes complicates *Cushing's syndrome acromegaly gigantism adrenal cortical tumor* or *hyperplasia, adrenal medullary tumor* and *arrhenoblastoma*

*Hyperthyroidism* usually shows a characteristic curve with a tendency to a quick high rise and a relatively early fall This will cause glycosuria but such patients may not be true diabetics and frequently cure of the hyperthyroidism will cause a disappearance of both the tendency to elevated blood glucose and the glycosuria Recently reported observations by Berger[3] indicate that administration of 100 units of corticotropin (ACTH) 1 hour before the glucose tolerance test causes higher blood sugar levels in patients who are diabetics and also in siblings of known diabetics whose previous control glucose tolerance curves have been normal He offers the interesting suggestion that in the latter group potential diabetics may be discovered by this means who would otherwise be missed

*Low glucose tolerance curves* (high sugar tolerance) are seen classically in patients with *hyperinsulinism,* particularly with a beta cell tumor of the pancreas With a *mild hypoglycemic* tendency it is important to run the glucose tolerance test for 5 or 6 hours as it may require that length of time for the hypoglycemic dip to be manifest Also when testing for this tendency it may be necessary to take an extra blood sugar not on the routine schedule when the patient develops signs or symptoms suggesting hypoglycemia This may indicate a hypoglycemic fall in the curve which will return to more normal levels later as glycogen is freed from the liver The tendency to hypo

glycemia is seen with varying intensities in *Simmonds disease* and in *Addison s disease* In both of these conditions it may be very marked and deaths from hypoglycemia have been reported

*Relatively low flat glucose tolerance curves* are found in *pituitary infantilism myxedema* and *cretinism*

## THE INSULIN TOLERANCE TEST

This is used occasionally as a test for diminished function of the anterior pituitary and demonstrates the patient s degree of sensitivity to insulin

**Method** A fasting blood sugar is taken and then a dose of 1/10 unit of regular insulin per kg of ideal body weight is given intravenously Blood sugar level is again tested 20 30 45 60 90 and 120 minutes after the injection *Normally* the level of blood sugar will fall to about 50% of the fasting level in 20 to 30 minutes and then will rebound to the fasting level in 120 minutes

*Hypoglycemic reactions* can occur with this test particularly if the patient has *hyperinsulinism Simmond s disease* or *Addison s disease* If any of these conditions are suspected it is prudent to use only one half to one third of the recommended dose of insulin and also to have glucose ready for intravenous injection in case a hypoglycemic crisis appears

## PLASMA CHOLESTEROL

The level of the plasma cholesterol is occasionally helpful as additional information in estimation of thyroid function

**Method** Venous blood is drawn and placed in an oxalate tube One cc of plasma is transferred to a 25 cc volumetric flask and 15 cc of a mixture of equal parts of

95% alcohol and acetone is added in a rapid stream while keeping the plasma in motion A finely suspended precipitate results The flask is placed in boiling water being kept in motion to avoid bumping until the solvent boils It is cooled to room temperature and the volume made up to 25 cc with the alcohol acetone mixture This is stoppered mixed thoroughly and filtered into a dry test tube (a watch glass over the funnel will minimize evaporation of the solvent) A clear filtrate results

*Saponification and precipitation* is the next step and for this two to three drops of potassium hydroxide solution (10 gm of reagent grade potassium hydroxide in 20 cc of distilled water) are placed in a 15 cc graduated centrifuge tube and 3 cc of the clear filtrate added This is mixed by drawing up and down in the pipette until no droplets of alkali can be seen The tube is then placed in a preserving jar containing a layer of sand about 3 cm deep previously heated in a water bath to 45 C The jar is tightly closed and placed in an incubator at 37 to 40 C for 30 minutes The tube is then allowed to cool to room temperature and the acetone alcohol mixture added to the 6 cc mark One drop of phenolphthalein solution (1 gm of phenolphthalein diluted to 100 cc with 95% alcohol) is added followed by 10% acetic acid drop by drop with mixing until the red color disappears One drop in excess is added followed by 3 cc of digitonin solution (500 $m_g$ of digitonin dissolved in 100 cc of 50% alcohol) and then by thorough mixing The tube is then placed in a preserving jar covered tightly and allowed to stand at room temperature for at least three hours After standing it is centrifuged for 15 minutes at 2800 r p m following which the supernatant fluid is decanted and drained in an inverted position The walls of the tube are then washed with 2 to 3 cc of ether the precipitate mixed and again centrifuged for 5 minutes at 2000 r p m

*Development of the color* is the next step For this a

shallow pan containing a layer of sand 3 cm deep is heated to 110 to 115 C in an oven The tube containing the washed precipitate is placed in the pan and left in the oven for 10 minutes Then while the tube is still in the hot sand 2 cc of glacial acetic acid is added in such a manner that it washes the walls of the tube This is well mixed left in the hot sand for an additional two minutes and then removed and cooled to room temperature After this the tube is placed in a water bath at 25 C with light excluded and allowed to come to temperature equilibrium Four cc of cold acetic anhydride sulfuric acid reagent is added mixed and the tube returned to the bath for 27 minutes It is then transferred to an Evelyn tube and read within the next 10 minutes (The acetic anhydride sulfuric acid reagent is prepared just before use Twenty cc of acetic anhydride is placed in a glass stoppered cylinder and chilled in ice water One cc of concentrated sulfuric acid is added gradually with mixing and cooling The mixture is stoppered shaken vigorously and kept in an ice bath It should be used within one hour)

A standard solution of cholesterol for comparison is prepared by dissolving dry cholesterol in glacial acetic acid This stable stock solution should contain 1 mg of cholesterol per cc It is kept in a cool temperature Before use it is warmed to room temperature and diluted with glacial acetic acid so that the dilute standard will contain 0 2 mg cholesterol in 2 cc This dilute standard is stable for about two months

In reading the color 2 cc of the dilute standard solution is placed in a centrifuge tube This also is left in the water bath at 25 C and allowed to come to temperature When ready 4 cc of the cold acetic anhydride sulfuric acid reagent is added This is mixed and returned to the bath for 27 minutes then transferred to an Evelyn tube and read within 10 minutes A blank is similarly pre

pared except that 2 cc of acetic acid alone is used instead of the standard cholesterol solution The specimens are read in a photoelectric colorimeter using a 635 filter and 6 cc aperture Calculation is as follows

$$\frac{\text{Density of unknown} \times 0.2 \times 100}{\text{Density of standard} \times 0.12} = \text{Total cholesterol in mg \%}$$

There is some difference of opinion concerning the normal range In some texts this is said to be from 150 to 190 mg % while other authorities believe that the level is to be considered normal anywhere between 100 and 300 mg % It is of some value to become familiar with the results of the test as performed in one laboratory or preferably even by one technician In the experience of the writer most normal patients have levels between 150 and 225 mg %

With lowered thyroid function (*hypothyroidism myxedema*) the values tend to be *high* (300 to 1 000 mg %) while with increased thyroid function (*hyperthyroidism exophthalmic goiter*) the cholesterol levels are *low or low normal* The test is not as dependable as the basal metabolic rate or blood iodine but occasionally provides helpful corroborative evidence and may be especially valuable in following the effects of treatment Furthermore it is useful in children who cannot cooperate properly in the performance of the basal metabolic test If thyroid substance is being administered and the cholesterol is still above the normal range the dose of thyroid should probably be increased If a patient with apparent myxedema is found to have a normal cholesterol the possibility of primary pituitary rather than thyroid myxedema must be considered This is of considerable importance as the two types of myxedema should be handled differently Administration of thyroid to a patient with pituitary myxedema

may precipitate an adrenal cortical crisis and fatality The cautious use of small doses is imperative

*High levels of plasma cholesterol* are also seen in *diabetes mellitus* particularly in the presence of acidosis and in *xanthomatosis* *slightly high levels* are found in *Cushing s syndrome* *adrenal cortical tumors* and *virilism* *Slightly low levels* may be found in *pituitary infantilism* *hyperinsulinism* and in *Addison s disease*

The recent work of Gofman and colleagues* in fractionating the serum lipids by observing their flotation in the ultra-centrifuge has aroused considerable interest and discussion It is suggested that the giant molecules of the cholesterol complex are associated with the development of atherosclerosis Accumulation of correlative clinical data is proceeding and is awaited with great interest

## BLOOD IODINE

Estimation of the protein bound iodine content of the blood as a test for thyroid function is being employed with increasing frequency This fraction is generally considered to be the hormonal iodine and thus the test should be a highly accurate measure of the amount of circulating thyroid hormone Within certain limitations this is true However the test is delicate very sensitive and technically difficult The results are expressed in micrograms rather than milligrams and the iodine itself acts as a catalyst in the reaction so is not the measured end point There may be considerable difficulty in setting up the test but once it is working well it is usually reliable

**Method.** A modification of that reported by Salter and McKay is used

One cc of the unknown blood serum is placed in an ashing tube and diluted with 2 cc of distilled water 0 5 cc of 10% zinc sulfate and after mixing 0 5 cc of

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Method A modification of that reported by Salter and McKay[7] is used

One cc of the unknown blood serum is placed in an ashing tube and diluted with 2 cc of distilled water 0 5 cc of 10% zinc sulfate and after mixing 0 5 cc of

0.5 N sodium hydroxide. This is mixed and allowed to stand for one half hour and then the tube is centrifuged until the sediment is well packed. This is washed with 2 cc. of distilled water and recentrifuged. The supernatant fluid is discarded and 1 cc. of 1 N sodium carbonate is added to the sediment. The tube is then placed in a drying oven for 48 hours at 110° C. or until dry. The sample is ashed in a muffle furnace at 680° C. for 2 hours.

The *determination* is made by adding 4 cc. of 1 N hydrochloric acid. The sides of the tube are scraped down with a glass rod and after effervescence has ceased 2 cc. of 7 N sulfuric acid and 1 cc. of distilled water are added. After mixing, the contents are poured into a centrifuge tube and centrifuged until the sediment is well packed. Two colorimeter tubes are then used—to one of which is added 1 cc. of distilled water. A 3 cc. aliquot of the supernatant fluid of the unknown is pipetted into each colorimeter tube and 0.5 cc. of 0.1 N sodium arsenite solution is also added to each tube. The colorimeter tubes and a 0.1 N ceric ammonium sulfate solution in 1.6 N sulfuric acid are placed in a constant temperature water bath at 39° C. for one hour or until up to temperature.

The amount of iodine is proportional to the rate of the reaction between the ceric ammonium sulfate and the sodium arsenite which causes the bright yellow color of the ceric ammonium sulfate to disappear. One cc. of ceric ammonium sulfate solution is added to each tube and readings are taken at 6 and 12 minutes. The reaction rates are plotted on semi-log paper with colorimeter readings plotted against time in minutes. Calculation is then made by the following formula:

$$X = \frac{a \cdot t}{t_1 - t}$$

a equals known amount of iodine added to colorimeter tube.
t equals time in minutes at intercept of the rate of the tube containing no extra iodine with designated horizontal.

t equals time in minutes at intercept of the rate of the tube containing the added iodine with the same designated horizontal

Since the colorimeter tube contains a 3 cc aliquot of the 7 cc sample the concentration of the iodine in the unknown serum will be $X \times 7/3 \times 100$ (micrograms %) The *normal range* in our experience is 4 to 8 micrograms %

With *hyperthyroidism* the values are *above the normal range* and with *hypothyroidism* they are *below it* Any iodine containing medications (including thyroid) must be stopped for four to five days before the day of the test No iodine should be used on the skin for sterilization at the time the blood is drawn If any organic iodine is given as for a cholecystogram intravenous pyelogram or myelogram the values will be high for a period of weeks to months—thus rendering the test valueless as an indication of level of thyroid function

Blood iodine determinations are as yet done in fairly few laboratories so that the available ones are frequently overworked as a consequence one is often delayed in learning the results of an individual test These factors of limited availability and slow reporting will undoubtedly be gradually eliminated particularly if a simpler method is developed Until this occurs however measurement of the basal metabolic rate will not be supplanted by the blood iodine test Finally the test seems to be a better gauge of hyper than hypothyroidism Furthermore the narrow range in micrograms is to be contrasted with the far more striking range of percentage in basal rate (10 to 50% minus in hypothyroidism and 10 to 100% plus in hyperthyroidism)

## SERUM CAROTIN

An increase in this factor is occasionally of interest in hypothyroidism

Method An equal volume of serum is mixed with 95% alcohol To this an equal volume of petroleum ether is added This is mixed by shaking to extract the carotin and allowed to stand for layering A yellow color in the upper (petroleum ether) layer indicates an excess of carotin in the serum The result may be reported as positive or the intensity of the color may be roughly indicated as 1 2 3 or 4 plus

*Carotinemia* (increased blood carotin) has been found to be present in most patients with *untreated myxedema* It is also found occasionally in other conditions associated with low basal metabolic rates including *Simmonds disease* and *hypogonadism* A dietary cause for the carotinemia (excessive intake of carotin containing foods) must be excluded

## SERUM CALCIUM PHOSPHORUS AND ALKALINE PHOSPHATASE

These can be considered together and are of special importance in the diagnosis of diseases of the parathyroids or other metabolic bone diseases

Method for serum calcium Two cc of serum are placed in a 15 cc graduated centrifuge tube containing 2 cc of water (The outside diameter of the tube should be 6 or 7 mm at the 0 1 cc mark ) One cc of saturated ammonium oxalate solution is added (4 2 gm per 100 cc of water) These are mixed with a circular motion and the mixture allowed to stand for at least 4 hours The contents are again mixed and then the tube is centrifuged at 1500 r p m for 10 minutes The supernatant is poured off and 2% ammonium hydroxide is added up to 4 cc in such a manner that it washes down the sides of the tube This is mixed by a circular motion until the precipitate spirals up in the center (all need not be mixed) The tube is then

centrifuged for five minutes and the supernatant again removed by decantation Following this the crystals are dissolved in 2 cc of approximately normal sulfuric acid and the tube and contents heated in a boiling water bath for two minutes Titration is then performed immediately with N/100 potassium permanganate to a definite pink color that persists for at least one minute

A blank is prepared fresh each day in order to standardize the permanganate solution For this 2 cc of N/100 sodium oxalate is mixed with 2 cc of normal sulfuric acid heated in boiling water for two minutes and then titrated with the N/100 potassium permanganate Then

$$\frac{2 \text{ cc (sodium oxalate)}}{\text{— cc } KMnO} \text{ equals correction factor}$$

Final calculation is then performed as follows

$$\frac{\text{— cc KMnO used on unknown} \times \text{Correction factor} \times 0\,2 \times 100}{2 \text{ (cc serum used)}} \text{ equals serum calcium in mg per 100 cc of serum}$$

*The normal range is 9 to 11 mg %* (However it is also important to know the level of the total serum protein for if this is low a greater percentage of the total calcium will be in the diffusible form—e g with a total protein of 4 gm % a serum calcium of 7 mg % would be normal)

**Method for serum inorganic phosphorus** In drawing the blood precautions are taken to prevent hemolysis One cc of serum is placed in a 25 cc volumetric flask with 5 to 6 cc of distilled water Four cc of 40% tri chloracetic acid is added and the flask filled to the 25 cc mark A pinch of kaolin is added and after standing for 10 minutes the mixture is filtered directly into 2 test tubes measuring volumetrically so that there is a 10 cc aliquot in each tube Two cc of a molybdate solution is added to each tube (This solution is prepared by dis solving 12 5 gm of ammonium molybdate in water dilut ing to 100 cc and then mixing in a 50 cc glass stoppered bottle with 150 cc of 10 N sulfuric acid This is brought

to volume and is reliable for 6 months) Next 0 8 cc of sulfuric reagent is added to each tube and after standing for exactly 20 minutes the solution is transferred to an Evelyn tube and read using a 660 filter in a photoelectric colorimeter (The sulfuric reagent is prepared by dissolving 200 mg of aminonaphtholsulfonic acid in 78 cc of 15% sodium bisulfate and 1 cc of 20% sodium sulfate After stoppering and shaking more of the sodium sulfate may be added in 1 cc amounts until the reagent is clear This is reliable for two weeks)

A blank is set up each time measuring directly into a test tube 7 cc of water 3 cc of 20% trichloracetic acid and mixing Two cc of a molybdate solution is then added (prepared similarly to the above except that 250 cc of 10 N sulfuric acid is used instead of 150 cc) After mixing 0 8 cc of the sulfuric reagent is added and the mixture allowed to stand for exactly 20 minutes It is then transferred to an Evelyn tube and the colorimeter adjusted to give 100% transmission

The serum phosphorus value is then read using the unknown and reading directly *from a graph with curve made up from standard solutions of potassium dihydrogen phosphate*

*The normal values for serum inorganic phosphorus in this laboratory are 3 to 4 mg % for adults and up to 5 mg for children*

**Method for alkaline phosphatase** One half cc of unhemolyzed serum *is placed in a 25 cc volumetric flask with 10 cc of alkaline substrate at 37 C* and then incubated for one hour at 37 C (The alkaline substrate is prepared by adding exactly 1 06 gm of sodium barbital to 1 25 gm sodium betaglycerophosphate and diluting to volume in a 250 cc volumetric flask Six drops of chloroform are added The substrate is reliable for one month if continuously refrigerated Before using the pH must be tested at 37 C after incubation for one hour at 37 C —it should be pH 9 3 ± 0 15)

After incubation the mixture is cooled under tap water and 4 cc of 40% trichloracetic acid is added The solution is brought to volume (25 cc) a pinch of kaolin added and after standing for 10 minutes it is filtered directly into two test tubes with a 10 cc aliquot in each tube Two cc of the molybdate solution (prepared as on page 25) is added to each tube and after mixing 0 8 cc of the sulfuric reagent is added (prepared as on page 26) This is allowed to stand for exactly 20 minutes and then transferred to an Evelyn tube and read in a photoeletric colorimeter using a 660 filter This result is then read directly from the phosphorus curve as described on page 26 A serum phosphorus determination must be performed simultaneously and the value for the phosphatase is obtained by subtracting the result of the phosphorus test from that obtained with the phosphatase test multiplying the phosphatase by two before the subtraction (to correct for the smaller amount of serum used in the phosphatase test)

*The normal values in this laboratory are from 0 to 5 units* Since the method was originally described by Shinowara Jones and Reinhart[8] these are frequently referred to as S J and R units and are comparable to the more widely known Bodansky units

The *blood calcium is high* (usually from 11 to 16 mg %) in *hyperparathyroidism* and in *vitamin D intoxication*—and may be *slightly high* in *Paget s disease* of bone It is *low* (usually under 7 mg %) in *hypoparathyroidism* (tetany) and has been found to be *moderately low* in *Simmonds disease Addison s disease* and in *eunuchoidism*

The *blood phosphorus is high* (usually from 4 to 10 mg %) in *hypoparathyroidism* (tetany) and may be *slightly high in diabetes mellitus acromegaly* and *Addisonian crisis* It is *low* (usually under 3 mg %) in *hyperparathyroidism* and is *slightly low* in *hyperinsulinism*

There is suggestive evidence that the level of *serum phosphorus* may indicate the amount of circulating *anterior pituitary growth hormone* A level of over 4 mg % has been considered evidence of an increased amount of growth hormone as in the active stages of *acromegaly* and *gigantism* This finding is still being confirmed

The *alkaline phosphatase* is *increased* specifically in *Paget s disease of bone* and is of diagnostic importance in this condition It is also increased if bone is involved in *hyperparathyroidism* (up to 20 S J & R units) but may be *low* in that condition if there is no activity in the bones It is moderately increased in *Albrights syndrome* (polyostotic fibrous dysplasia) In *osteoporosis* particularly the senile or menopausal types the blood calcium phosphorus and alkaline phosphatase are usually within normal limits

The *acid phosphatase* has been reported to be elevated in some patients with *carcinoma of the prostate* especially if there is metastatic involvement of bone

## SERUM ELECTROLYTES (SODIUM POTASSIUM CHLORIDE)

Study of blood levels of these constituents are particularly important in diseases of the adrenal cortex These may be primary or secondary to disease of other endocrine glands Adrenal cortical dysfunction may also result from non endocrine diseases or Stress

**Serum sodium method** A Perkins Elmer flame photometer is used in our laboratory This is an emission spectrometer combined with a flame source and a photo electric detection system for the measurement of intensity of light The unknown serum is diluted 1/100 and a lithium nitriate solution (made by dissolving 13 9 gm of lithium nitrate in 2 liters of water) is diluted 1/10 The latter is used as an internal standard with the light of

the unknown element being compared directly to the light emitted by the element lithium Standard solutions are used for comparison and the results read on a calibrated curve showing the milliequivalents per liter The intermediate standard solution is prepared by adding 30 cc of a stock solution (made up by dissolving 2 54 gm of NaCl in 1 liter of water) to 100 cc of the lithium nitrate solution and making up to 1000 cc with water A 100% standard is made up by adding 25 cc of the stock solution to 50 cc of the lithium solution and making up to 500 cc with water The *unknown* is prepared by adding 1 cc of serum to 10 cc of the lithium nitrate solution and making up to 100 cc with water

**Serum potassium method.** This is also accomplished with the Perkins Elmer flame photometer The unknown serum is diluted 1/10 and the lithium nitrate solution (prepared as above) is diluted 1/10 and used as the internal standard Standard solutions are used for comparison and the results read on a calibrated curve showing milliequivalents per liter

Standard solutions are made up from a stock solution containing 1 91 gm of KCl per liter The intermediate standard is prepared by adding 20 cc of the stock solution to 100 cc of the lithium nitrate solution and making up to 1000 cc with water The 100% standard is made up by adding 25 cc of the stock solution to 50 cc of lithium nitrate solution and making up to 500 cc with water The *unknown* is prepared by mixing 1 cc of serum with 1 cc of the lithium nitrate solution and making up to 10 cc with water

**Serum chloride method** A standard 4 mM (milliequivalent) chloride solution is prepared by mixing 10 cc. of 0 1 N NaCl solution with 250 cc of a phosphoric tungstic acid reagent (prepared by dissolving 6 gm Na WO 2H O reagent grade in 1 liter of 0 15 M H PO ) It is standardized by shaking 25 cc with 0 3 gm precipitated silver iodate for 40 seconds and then centrifuged in a capped

tube A 20 cc. portion of the clear supernatant is placed in a 100 cc Erlenmeyer flask 2 gm sodium iodide is added and then titration is performed with 0.02303 N sodium thiosulfate solution adding 3 or 4 drops of 1% starch solution when the yellow iodine color has nearly disappeared The titration should take 20.8 cc of the thiosulfate solution and if deviation is more than 0.50 cc a correction factor must be used

With the *unknown serum* 0.2 cc. of the serum is added to 5 cc. of the phosphoric tungstic acid reagent (preparation described above) in a test tube One glass spoon (60 mg ± 10 mg) of silver iodate is added and the mixture shaken for 40 seconds and filtered A 2 cc aliquot of the filtrate is titrated with the 0.02303 N sodium thiosulfate solution after adding 0.2 gm sodium iodide (one glass spoon) using a 50 cc Erlenmeyer flask Three or four drops of 1% starch solution are added when the yellow iodine color has nearly disappeared as an aid in arriving at a clear end point

*Calculation* is as follows

*Milliequivalents of chloride per liter of serum* = 50 × cc 0.02303 N sodium thiosulfate used

*Grams of chloride per liter of serum* = 2.923 × cc of 0.02303 N sodium thiosulfate solution used

If a correction factor is indicated by titration of the standard chloride solution as mentioned above it must be included in the calculation

Note that the results are expressed in milliequivalents rather than in milligrams % Comparative normal values are as follows

| | mg % | *Milliequivalents* |
|---|---|---|
| *Serum Sodium* | 360 mg % | – 142 m/equiv – (normal range 136 to 142 m/equiv ) |
| *Serum Chlorides* | 375 to 480 mg % | – 103 m/equiv – (normal range 99.5 to 106.6 m/equiv ) |

*Serum chlorides as NaCl* 5.0 to 6.0 mg %

*Serum Potassium* 20 mg % — 3.5 to 5.3 m/equiv

Results expressed in milliequivalents are appearing with increasing frequency from clinical laboratories and the change seems justified The milliequivalent figures are simpler and easier to remember and can be compared with other values including the $CO_2$ combining power similarly expressed

The following formulae have been found useful[9]

$$\frac{\text{Na Cl mg \%}}{58.5} = \text{Cl m/equiv/L}$$

$$\frac{CO_2 \text{ vol \%}}{3} = HCO_3 \text{ m/equiv/L}$$

$$\text{Cl m/equiv/L} + CO_2 \text{ m/equiv/L} + 10 = \text{Na m/equiv/L}$$

$$\text{Cl m/equiv/L} + 23.2 + (0.5\ HCO_3) = \text{Na m/equiv/L}$$ [10]

It would appear advantageous to have standardization of reports in milliequivalents rather than milligrams % especially with ions involved in the electrolyte balance

*In adrenal cortical deficiencies* blood levels of *Na and Cl are low* and the K *content* is *high* The *opposite* may occur in *Cushing's syndrome* and also occasionally in *acromegaly* Similar changes i e *slightly elevated Na and Cl and slightly low K* are encountered in *adrenal cortical virilism adrenal precocious puberty* and *arrhenoblastoma* In *Simmonds disease* the factor of *adrenal insufficiency* may be pronounced and the electrolyte changes are similar to those in Addison's disease with *low values for sodium and chloride* and a *high potassium level In hyperthyroidism* the blood *sodium* is occasionally *low* and conversely may be *slightly* high in *hypothyroidism*

## NITROGEN METABOLISM

Several tests of the blood may give information regarding the nitrogen metabolism These include the blood uric acid, blood urea nitrogen and the non protein nitrogen The latter is the most widely used and reported Methods for testing for these components are available in any laboratory text The *non protein nitrogen* has been found to be *elevated* in several endocrine conditions, including *Addisonian crisis, terminal Cushing's syndrome, terminal hyperparathyroidism* and *diabetic coma* The *blood uric acid* is *elevated* specifically during active attacks of *gout*

## PLASMA $CO_2$ COMBINING POWER

This is a test specifically for acidosis or alkalosis of various origins As with the electrolytes it is becoming increasingly frequent to report results in milliequivalents With the older manner of reporting the normal range was 50 to 70 volumes % However with milliequivalents the average result is 28 m/equiv with a normal range of 24 to 30 m/equiv

Method For this test the manometric apparatus as described by Peters and Van Slyke is used[11] The procedure as outlined by these writers on pages 283 to 286 of the reference is followed exactly in our laboratory Blood is drawn and centrifuged anaerobically for the test

The results are *low* in *diabetic acidosis* or acidosis from other causes and are *occasionally low* in *adrenal cortical insufficiency* It may be *slightly high* in *parathyroid tetany*

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*Chapter IV*

# SPECIAL TESTS OF URINE (EXCLUDING HORMONE ASSAYS)

## URINE CHLORIDES

EXCRETION of chlorides is *increased* in *Addison's disease*, which fact is the basis of several tests for adrenal cortical hypofunction.

The *Cutler Power Wilder test* is one of these and has been available since 1938.[1]

Method. The test takes three days and hospitalization is necessary. Treatment with adrenal steroids or extra salt is withheld the day preceding. A low salt diet (containing 0.95 mg. of chloride ion, 0.59 gm. of sodium and 4.1 gm. of potassium per day) is started on the first day and free drinking of water is encouraged. On the afternoon of the first day extra potassium is given as potassium citrate 33 mg. per kg. body weight (42 mg. of potassium citrate per lb.).

*Second day*—Fluid intake to 40 cc. per kg. of body weight is given, and potassium citrate is repeated. Urine is collected from 8 a.m. to 8 p.m.—and a second sample from 8 p.m. to 8 a.m. of the third day.

*Third day*—Twenty cc. of liquid per kg. of body weight is given before 11 a.m. and a third urine sample is collected from 8 a.m. to 12 noon.

The urine samples are then examined for chloride content, the *most significant being the four hour sample collected on the third day. In this specimen the normal controls showed a mean of 54.2 mg.% with a range of 17 to 141 mg.%, while patients with Addison's disease*

*showed the increased mean value of 29? 4 mg % with a range of 229 to 356 mg %*

The *obvious disadvantages* of this test are the necessity for hospitalization the length of time required (three days with one day of preparation) and the danger of with holding treatment and putting a patient with Addison s disease on a low salt diet with high potassium intake The latter can induce an Addisonian crisis and facilities must be at hand for immediate supportive treatment to prevent possible fatal outcome—considerable risk for a laboratory procedure

The *Robinson Power Kepler water test* described in 1946 [2] is simpler and safer and has largely replaced the older procedure

The method consists of two sections the first being de pendent upon the inability of patients with Addison s disease to excrete water normally because of the decreased glomerular filtration rate The second section gives data on the urea and chloride clearance in the patient with the first usually being reduced and the second increased The urea chloride ratio points up this abnormality It is not always necessary to perform the second portion and the test can be completed in one day in either case Also it can be accomplished as an office procedure provided a place is available for the patient to rest

**Method** *First portion*—On the day before the patient omits extra salt and takes no food or fluid after 6 p m At 10 30 p m the bladder is emptied and the urine dis carded All urine voided from then until 7 30 a m is collected and this *night volume measured* No breakfast is given and the patient is asked to void at 8 30 a m fol lowing which he is given 20 cc of water per kg of body weight (9 cc per lb ) to drink within 45 minutes Urine specimens are collected at 9 30 10 30 11 30 a m and

12 30 p m (with the patient kept at rest) and the volume of each is measured separately

If the volume of any of these day specimens is greater than that of the night specimen the patient probably does not have Addison s disease and the test can be terminated However if none of the day volumes equal that of the night specimen the second part of the test is performed

*Second portion*—A specimen of blood is taken while the patient is still fasting (at 12 30 p m ) and chemical analyses are made to set up the following equation

$$\frac{\text{Urine Urea Night spec mg \%}}{\text{Plasma urea mg \%}} \times \frac{\text{Plasma chloride mg \%}}{\text{Urine chloride night spec (mg \%)}} \times \frac{\text{Volume of largest hourly urine spec (cc)}}{\text{Volume of night urine (cc)}} = \text{A}$$

When the above equation has been resolved the factor A will be 30 or above in normal patients and 25 or below in patients with Addison s disease

The test is a good one but still only gives a probable answer and in some instances repetition may give contradictory results If the outcome is equivocal the Cutler Power Wilder chloride excretion test can be done immediately following—using this water test as the first day of the other and following the rest of the procedure as outlined above However the more recent availability of the eosinophile count and corticotropin (ACTH) tests (Chapter III pp 10 11) has largely replaced the dangerous chloride excretion test

An excellent survey of the present status of tests for adrenal cortical function can be found in a recent paper by Thorn *et al*[3]

## THE CARTER ROBBINS TEST[4]

This test is useful in distinguishing true *diabetes insipidus* from psychogenic diuresis and has as its basis test

ing the excretory function of the kidney It is somewhat complicated but may provide the answer to a difficult clinical decision

**Method** Antidiuretic therapy is stopped long enough for the patient s polyuria and polydipsia to reappear Then fluids are withheld for eight hours but food is per mitted Just before the test the patient is given 20 cc of water per kg of body weight to drink in one hour Thirty minutes after the start of hydration an indwelling catheter is inserted and urine specimens collected in 15 minute periods calculating the urine flow in cc per minute After two control periods with adequate flow (at least 5 cc per minute) an intravenous infusion of hypertonic saline (2 5%) is started and administered for 45 minutes at the rate of 0 25 cc per kg per minute If there is no decrease in the flow of urine during the infusion or during the first two post infusion periods of 15 minutes each 0 1 unit of pitressin is given intravenously and the effect on the rate of urine flow observed

Normal patients show a prompt and marked decrease in urine flow after the start of the hypertonic infusion while patients with true *diabetes insipidus do not show any de crease until the pitressin is administered*

This test is not necessary in most cases of diabetes in sipidus but occasionally can be helpful when differentia tion from psychogenic polydipsia is uncertain

## URINE CONCENTRATION TEST

This test is simpler and is occasionally helpful in the diagnosis of *diabetes insipidus*

**Method** A simple modification is to collect the fasting urine specimen and then at 8 a m to have the patient drink one liter of water within 1/2 hour Urine specimens then are collected at hourly intervals until 5 p m allow

ing the patient no fluids and only a dry luncheon during this time The volume and specific gravity of each specimen is measured Normally the specific gravity rises as the day progresses often reaching 1 022 to 1 032 The commonly used Fishberg modification of the Volhard test which requires abstinence from any fluid for a day and a night and part of the next day may be impossible in a patient with diabetes insipidus because of the excessive thirst characteristic of the disease

In *diabetes insipidus* the urine does not concentrate to above 1 001 or 1 005 The test may be difficult to perform because of intense discomfort resulting from great thirst during the period of withholding fluid

## SULKOWITCH TEST

This is a simple qualitative test for the amount of calcium in the urine It is satisfactory and of considerable help clinically in diseases with deranged calcium metabolism The test furnishes a quick and surprisingly good indirect estimate of the level of the blood calcium

*Method* Five cc of Sulkowitch reagent are added to about 5 cc. of centrifuged urine The density of the cloud is noted after mixing and allowing to stand for two minutes Results are recorded as follows

0 —clear
1 plus—slight cloud
2 plus—moderate cloud
3 plus—heavy cloud
4 plus—solid cloud

Normally 1 plus 2 plus or occasionally 3 plus are seen depending upon the amount of calcium in the diet

If the result is 0 hypocalcemia is present (usually under 7 5 mg %) as in tetany If the result is 4 plus or occasionally even 3 plus hypercalcemia is indicated (usual

ly over 11 mg %) as in hyperparathyroidism The Sulkowitch reagent is an oxalate buffer solution and is prepared as follows

| | |
|---|---|
| Oxalic acid | 2 5 gm |
| Ammonium oxalate | 2.5 gm |
| Glacial acetic acid | 5 0 cc. |
| Distilled Water q.s ad | 150 0 cc. |

Some authors suggest that the test be done on a 24 hour urine specimen However if a morning fasting urine specimen is used it is less likely to be influenced by dietary calcium intake or amount of fluid ingested—and can be compared from day to day in following the effects of therapy In some laboratories the Sulkowitch is part of the routine urinalysis thus possibly furnishing a diagnostic clue to an unsuspected instance of hyperparathyroidism or to other diseases with excessive bone destruction such as severe osteoporosis or Paget s disease

The test shows a *strongly positive* result in *hyperpara thyroidism* in active *Paget s disease of bone,* and in *severe osteoporosis* When the latter two conditions are under treatment with the sex steroids reduction in the intensity of the Sulkowitch reaction is an indication of the effective ness of treatment The test is *negative* or *low* in *para thyroid tetany* and may be of value in suggesting *hypo parathyroidism* (post operative) as a cause for some of the patient s symptoms which might otherwise be ascribed to hypothyroidism If vitamin D is being administered in large doses as for arthritis an increasingly positive Sulko witch test will give warning of beginning toxic effects

## PHOSPHORUS EXCRETION

Study of the *excretion* of *phosphorus* in the urine is performed occasionally especially in differentiating *hypo parathyroidism* from *pseudohypoparathyroidism* (the so-

called Sebright bantam syndrome—in which the system seems resistant to the effects of parathormone—an instance of end organ unresponsiveness) *The test of Ellsworth and Howard*[5] in which the hourly excretion of phosphorus for three hours before and three to five hours after the injection of 2 cc (200 units) of parathormone is determined In *pseudohypoparathyroidism* the excretion of phosphorus remains *constant or only slightly elevated*—while in true *hypoparathyroidism* the urinary phosphorus shows a *marked increase* after the injection

## ACETONE BODIES

Acetone and diacetic acid are easily tested for in the urine and in some laboratories one test or the other is a routine procedure They are *present* in *diabetic acidosis* or acidosis of other origin including starvation as when a too strenuous reduction regime is being pursued

## CREATINE IN URINE

This test is occasionally helpful in the diagnosis of male hypogonadism

Method A 24 hour urine specimen is collected The urine volume (V) is measured The specific gravity is measured and if above 1 010 the specimen is diluted to that level with water and the volume again measured (Vd) (only a small volume (i e 5 cc) need be diluted and corrections can be made for total dilution volume) Five cc are then again diluted to 100 cc in a volumetric flask If the original specific gravity is less than 1 010 no original dilution is necessary

One cc of the diluted urine is then mixed with 8 cc of N/12 sulfuric acid and 1 cc of 10% sodium tungstate This is thoroughly mixed by shaking and then filtered if necessary Eight cc of the filtrate is placed in an Evelyn colorimeter tube and the mouth covered with tinfoil This is

autoclaved for 20 minutes (115 to 120 C) at 15 lbs pressure and then allowed to cool

It is necessary to run a specimen for urine creatinine at the same time and in order to do this 1 cc of the diluted urine is prepared exactly as in the preceding paragraph except that it is not autoclaved

An alkaline picrate solution is then prepared by adding 1 volume of 10% sodium hydroxide to 5 parts of a picric acid solution (of which each liter contains 11 75 gm of pure picric acid) This should be used within 5 minutes Four cc are added to each of the tubes and after standing for 20 minutes for development of color the readings are made in a photoelectric colorimeter using a 515 filter A blank tube is used at first containing 8 cc of water to which also has been added 4 cc of the alkaline picrate solution and allowed to stand for 20 minutes The galvanometer is set at 50 so later readings are multiplied by two before conversion Conversion is accomplished by reference to a chart previously prepared using dilutions of a stock solution containing 1 mg of creatinine per cc

Calculations are as follows

1 Diluted volume of urine (Vd) × 100 × mg of creatinine (reading from chart)
――――――――――――――――――――――――――――― =
Original urine volume (V) × aliquot of diluted urine in cc

mg of creatinine per 100 cc —

2 Original urine volume (V) × mg of creatinine per 100 cc
――――――――――――――――――――――――――――― =
100

gm creatinine in total urine specimen

For *creatine*—the difference between the readings of the autoclaved and unautoclaved specimens represents creatine in terms of creatinine This is converted to creatine by multiplying by the factor 1 16 This conversion can be made after the first or second calculation above depending upon whether the mg per 100 cc of urine or the gm per total volume of urine is desired

*Normal values* for adults are 0 to 186 mg /24 hours and for children 10 to 15 mg /24 hours

Creatine is present normally in the urine of children and of adult women However it is very low or absent in adult men *In endocrine disease it is found to be present in adult men with hypogonadism* and occasionally with *hyperthyroidism* and *diabetes mellitus* It may be *absent in children* with *childhood myxedema* The evidence presented by the test is usually corroborative but not critical in arriving at a clinical diagnosis

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*Chapter V*

# BASAL METABOLIC RATE, RADIOACTIVE IODINE UPTAKE, GASTRIC ANALYSIS, ELECTROCARDIOGRAM, BLOOD PRESSURE TESTS

## BASAL METABOLIC RATE

ESTIMATION of the basal metabolic rate has been used for years in determining the level of thyroid function Recently the test has been the subject of criticism because of the variation in results and lack of specificity in comparison with the determination of the protein bound iodine However it has stood the test of time and if its limitations are realized and above all if there is good clinical correlation it is still of considerable value Also in its favor are the facts that it is relatively easy to perform and is widely available

**Method** This varies considerably depending upon the machine used so will not be described However the more common *factors of error* will be mentioned These include poor relaxation of the patient * perforated ear drum faulty functioning or leaks in the machine worn out soda lime faulty placing of the nasal clip so that leaks can occur and the patient not being in an absolute fasting state Emotional factors also may alter the result as has been brought out in recent studies of the *somnolent*

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* The rate does not change appreciably during the menstrual flow but discomfort associated with the period may interfere with proper relaxation of the patient and result in an unsatisfactory test Therefore it is well not to perform the test on patients who are experiencing menstrual molimina and probably never on the first day of the menstrual period

*metabolic rate* by Rapport Curtis and Simcox[1] They performed basal metabolic rates in the usual manner and then put the patient to sleep with intravenous Nembutal (50 mg then 25 mg per minute until asleep) The test was then repeated and the values were 7 to 10% lower than those taken while the patient was awake However in nervous patients the values were 30 to 34% lower

As is well known *in most normal patients* values are between minus 10% and plus 10% With *hyperthyroidism* the figures are *above plus 10%* and with *hypothyroidism* they are below *minus 10%* (Office tests usually average about 5% higher than those performed in the hospital) *Occasionally however an individual will be seen who has* a basal rate in the vicinity of minus 20% who feels perfectly well and who shows no signs of hypothyroidism Administration of thyroid substance in an amount sufficient to raise the rate to usual normal levels will result in *symptoms of over dosage so one must conclude that the* normal level for that individual is approximately minus 20% Also if this person should develop hyperthyroidism a basal rate of plus 10% may indicate a significant elevation Thus the need for clinical correlation is even more *apparent*

A *high basal rate* is seen occasionally in *leukemia* the writer having encountered one instance of plus 100% Of the endocrine diseases a *moderately high rate* is seen occasionally in *active acromegaly gigantism Cushing s syndrome tetany* also with *tumors of the adrenals gonads pineal gland* or with *arrhenoblastoma* Non endocrine causes of increased metabolic rate are also numerous and include pregnancy fever and diseases of several of the body systems A recent excellent review of the causes of extra thyroidal *hypermetabolism has been published by* Bruger and Hollander[2]

*Very low rates* are found in *Simmonds disease* with levels of minus 45% and minus 50% being reported Similar *low levels* are found in *anorexia nervosa* in which the clinical picture is almost identical although the fundamental causative factor is psychological rather than endocrine

*Moderately low basal rates* are found in the late burned out phases of acromegaly and gigantism They are also encountered in *infantilism* *Addison s disease* and *hypogonadism*

Other less specific tests of thyroid function including the specific dynamic action test the galactose tolerance test and the acetonitrile test are not in general use at this time

## RADIOACTIVE IODINE UPTAKE

The rate of uptake of a tracer dose of this material is occasionally of considerable help in the diagnosis of *hyperthyroidism* However it does require a setup for handling the radioactive material and measuring its concentration and is therefore available only in relatively few properly equipped centers

Method The patient must not have had Lugol s solution antithyroid drugs or any other source of iodine for two to four weeks before the test Organic iodine preparations as used for cholecystograms pyelograms or myelograms may interfere with the test for a longer period

On the morning of the test the patient is given 50 microcuries of $I^{131}$ by mouth and then allowed to eat breakfast Counter readings are taken over the neck and just above mid thigh (where the diameter is similar to the neck but without thyroid tissue) at one hour three hours and five hours A scintillation counter is used at a distance of 40 cm In constructing uptake curves the thigh reading is subtracted from the neck reading

*Normally* the uptake at five hours is from *5 to 30%* in the gland at the peak of a *gradual curve*

In *hyperthyroidism* the overactive gland takes up the $I^{131}$ much more rapidly and completely making a *steeper*

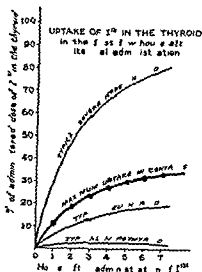



FIGURE 1 Radio iodine uptake studies in normals and in patients with *hyperthyroidism* and hypothyroidism Curves are average levels as individual tests show considerable spread on the graph (*Courtesy of Dr Earl R Miller*)

*curve* The 5 hour readings vary from 45 to 90% However any reading above 30% is considered to indicate possible hyperthyroidism and a search is made for clinical and other evidence of the disease

*Low levels* (i e 5 to 10% uptake at five hours) are *not as helpful* in the diagnosis of *hypothyroidism* and may be found *without this condition being present*

## GASTRIC ANALYSIS

The gastric acidity has been found to be low or absent in *Simmonds disease* and in *50%* of a series of proven cases *achlorhydria* was present It may occur also in *myxedema* and in *Addison s disease*

## ELECTROCARDIOGRAM

Electrocardiographic changes are occasionally seen in endocrine diseases and may be characteristic though not pathognomonic

In *myxedema* typical changes are *bradycardia prolonged P R interval low voltage with small complexes* and *changes in the S T segments* in the left precordial leads The *T waves* may be *depressed or inverted* and the changes can be compared with those seen in coronary disease All of these may return to normal as the patient improves under treatment with desiccated thyroid substance

In *Simmonds disease* the lowered metabolism may also result in *small complexes* and *bradycardia* occurs with some frequency

*Hyperthyroidism* may result in characteristic changes including *a rapid rate tall R waves tall T waves* and occasionally *auricular fibrillation* or *flutter* These arrhythmias may be paroxysmal in nature Later in the course of the disease signs of *left ventricular hypertrophy* may appear

In *late Cushing's disease* hypertensive cardiovascular disease may develop and signs of *left ventricular hypertrophy* and *myocardial damage* will appear

During attacks of hypertension with *pheochromocytoma rapid rate* and *extra systoles* may appear

Electrocardiographic changes can indicate *changes in the level of the blood potassium* and have been of considerable importance and help clinically In *diabetic coma* treatment with large amounts of fluids may cause an excessive diuresis of potassium to the extent that serious or even fatal potassium deficiency can occur This should always be suspected when the patient in diabetic coma is

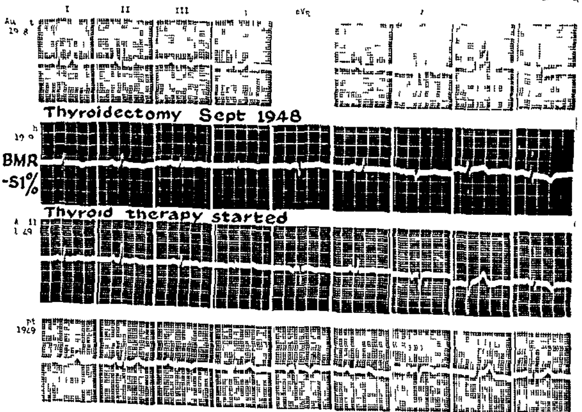



FIGURE 2 Electrocardiographic changes in post operative hypothyroidism Thyroidectomy in September 1918 and in March 1919 BMR was minus 51% Thyroid then started—note progressive improvement in T waves Patient was a female age 47 ... (Co tte y of Dr Ma e S kolow)

not responding properly to therapy In such circumstances when a determination of blood serum potassium is not easily available the electrocardiogram may be of considerable help in furnishing a quick estimate of the situation[3] As the blood *potassium falls* the *voltage de*

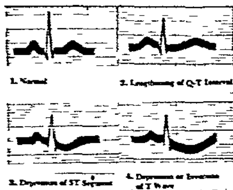



FIGURE 5 Electrocardiographic changes in progressive hypopotassemia (idealized). Knowledge of the rate is also helpful as a fast rate is characteristic of hypopotassemia, while similar changes may be encountered in hypocalcemia, with the difference that the rate is slow (After *Physician's Bulletin*—Eli Lilly & Co. *17* 46 May June 1952, courtesy of Dr D C. Hines)

*creases* the *T waves become flat* and a *prolonged Q T interval* develops If the level is *very low* the *T waves become inverted* the *S-T interval is depressed* and the *Q T interval is further prolonged* The *prolonged Q T interval is probably the most valuable* and characteristic of the changes In hyperpotassemia (potassium intoxication) progressive elevation of serum potassium causes tall peaked T waves widening of the QRS complexes depression of the S-T segments and finally auricular standstill with disappearance of P waves.

## BLOOD PRESSURE TESTS

Tests which influence the level of blood pressure may be of considerable value in the diagnosis of *adrenal medullary tumor (pheochromocytoma)* The most helpful is the *benzodioxane test* which lowers the pressure in this condition if it is elevated Recently Gifford Roth and Kvale[4] have reported the use of the *Regitine test* which also lowers the pressure and which they believe to be safer pleasanter and easier to administer The *Dibenamine test* also is occasionally helpful in the presence of an elevated blood pressure in ruling out pheochromocytoma If the blood pressure is normal the tests using *histamine* or *Mecholyl* or *tetraethylammonium* may be employed to induce a paroxysm The *cold pressor* test is also used occasionally to help rule out pheochromocytoma However these latter three tests which may induce a sudden rise in blood pressure carry the risk of inciting dangerous complications including central nervous system hemorrhage etc

**Methods** *Benzodioxane test* Goldenberg Snyder and Aranow[5] noted that the injection of piperidyl methyl benzodioxane in doses of 10 mg per sq meter of body surface given intravenously over a period of two minutes resulted in a fall of blood pressure lasting up to 15 minutes in the presence of pheochromocytoma The fall did not occur in other types of hypertension While the test has not proved uniformly reliable it is still a valuable one

*Regitine test*[4] This is performed by injecting 5 mg of Regitine (C-7337) intravenously In the presence of pheochromocytoma there is a marked fall in blood pressure if hypertension is present The blood pressure may drop in patients with essential hypertension but not to the same degree usually less than 35 mm mercury systolic and 25 mm diastolic

*Dibenamine test* Spear and Griswold[6] reported that following the administration of 7 mg of dibenamine per kg of body weight given intravenously in 300 cc of 5% glucose in normal saline solution over a period of one hour significant decrease of blood pressure occurred in patients with pheochromocytoma In patients with moderate essential hypertension the test usually induces only a slight postural hypotension

*Histamine test* Roth and Kvale[7] reported that in suspected cases of pheochromocytoma the injection of 0 05 mg of histamine base intravenously would induce an attack of hypertension usually within 2 minutes This was in contrast to a slight rise in normal individuals or patients with other types of hypertension

*Mecholyl test* Guarneri and Evans[8] suggested the use of Mecholyl giving 25 mg subcutaneously to induce a hypertensive attack in patients with adrenal medullary tumor They believed it to be more accurate than the histamine test

*Tetraethylammonium test* This is given intravenously in a dose of 400 mg In hypertension the blood pressure will fall markedly and with normal tension a mild hypotensive effect will result However with pheochromocytoma the injection will frequently (but not invariably) induce a hypertensive attack This lasts longer than that induced by histamine but can be controlled by placing the patient in the erect position Roth and Kvale[7] have reported experience with this test

*Cold pressor test* This test which is performed by immersing one of the patient s hands in ice water for 1 minute is not as likely to produce hypertension with pheochromocytoma as with other types of labile hypertension

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are open or closed An estimation can be made from the wrist film alone using the tables compiled by Shelton[1] and the Atlas of the hand by Todd—or by a more complete study of the joints of the extremities using the tables com-



FIGURE 4 Acromegalic skull—note enlarged sella turcica ballooning of frontal sinuses thickened calvarium elongated mandible with prognathism and prominent occipital protuberance (After R F Escamilla Diseases of the Pituitary in Musser Wohl *Textbook of Internal Medicine* 5th Edition Philadelphia Lea and Febiger 1951)

piled by Hodges[2] The second method is preferred by the writer as it gives a more complete picture of the develop ment and allows both the physician and the radiologist to estimate better the potentialities of further growth A good routine is one in which films are made of one elbow one wrist and hand pelvis including hips one knee one

ankle one foot and a lateral view of the skull If the patient is near bone maturity (beyond 16 to 17 years) it may be valuable to include the shoulder and clavicles

*Marked retardation* of the bone age is seen in *childhood myxedema* A good example of this was seen in a child cited personally to the writer by Dr C K Canelo of San Jose California This myxedematous child of two years had a bone age equivalent to that seen in the seventh month of fetal life Thus at two years the bone age was minus two months[1]

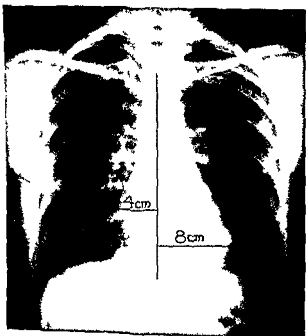
*Moderate retardation* is seen in *pituitary infantilism* and in *eunuchoidism* Surprisingly some *retardation* is occasionally seen in *gigantism* particularly if there is a factor of secondary eunuchoidism Mild retardation is the rule in cases of ovarian agenesis

*Advanced or accelerated bone age* well beyond the chronological age is seen in *pubertas praecox* of *adrenal, gonadal* or *pineal origin* Rapid premature closure of the epiphyseal growth zones accounts for the eventual short stature in these patients in spite of the spurt in growth to above normal levels (transitory gigantism) which usually characterizes the early stages of the disease

## DENTAL X RAYS

Studies of the teeth may show characteristic changes in certain of the endocrine diseases[3] In *hyperparathyroidism* there is characteristically a *disappearance of the lamina dura* around the roots of the teeth With *hypoparathyroidism* *enamel defects* including transverse furrows and horizontal grooves may be seen as well as *blunting of the roots* These changes occur particularly in the incisors and canines

*Increased spacing* between the teeth is seen in *acromegaly* and *gigantism* and *prognathism* with change in bite may



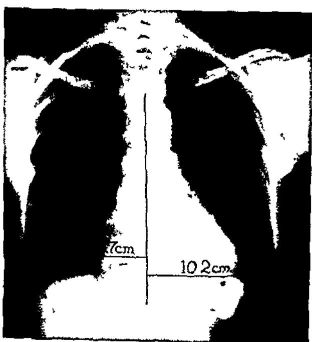




FIGURE 5 Chest x ray showing myxedema heart The enlarged flabby *atonic* heart returned to normal size under treatment with thyroid (After R F Escamilla H Lisser and H C Shepardson *Ann Int Med* 9 308 1935)

be apparent in acromegaly Conversely *crowding of the teeth* is frequent in *hypophyseal infantilism* because of the small dental arches

*Dental age* can be estimated in childhood by noting advance or delay in the usual sequence of eruption It is usually *retarded* with *hypothyroidism childhood myxedema* or *cretinism* or may be *advanced* with *precocious puberty*

Dental x rays also may emphasize the presence of *osteoporosis* The teeth do not lose their calcium readily while the maxillary and mandibular arches may participate in a generalized osteoporotic process so that the contrast between the two is particularly marked—a fact which may be helpful in diagnosis

## X RAY OF CHEST

This may demonstrate the *large flabby atonic heart* dilated in all its chambers which is characteristic of *myxedema* Occasionally a similar appearance is caused by an accumulation of *pericardial fluid* in the same disease and *pleural fluid* is also seen at times The heart also may be *enlarged* as part of the general splanchnomegaly of *acromegaly* and conversely may be of *small size* as part of the splanchnomicria which is characteristic of *advanced Simmonds disease* In *late Cushing's disease* and *late hyperparathyroidism* the development of hypertensive cardiovascular disease may result in *cardiac hypertrophy* This enlargement also is found occasionally in *late hyperthyroidism*

In *lateral view* chest films may demonstrate *acromegalic changes* such as a *prominence of the sternum* and *enlargement of the vertebrae* with *cervico dorsal kyphosis* In *Cushing s syndrome* the marked *osteoporosis* may result in *collapse of the vertebrae* causing the *typical kyphosis*

Chest films may reveal *tuberculosis* as the causative

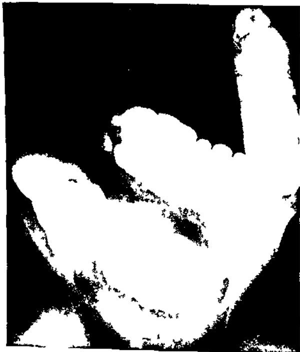


FIGURE 6 Colon in myxedema Films taken after voluntary emptying of bowel following barium enema Note *atonic bowel before treatment* and improved tone and emptying after treatment with thyroid (After R F Escamilla H Lisser and H C Shepardson *Ann Int Med* 9 310 1935)

factor in *Addison's disease* Evidence of *substernal goiter* also may be seen and occasionally in *precocious puberty* a *thymic tumor* is found

## X RAY OF ABDOMINAL REGION

Films may reveal *calcification* of the *adrenal glands* in *Addison's disease* Studies of the *gastrointestinal tract* occasionally demonstrate the *atony* and sluggish function characteristic of *myxedema* and studies of the *urinary tract* may also reveal *atony of the bladder* in this condition Other urinary tract findings include signs of *renal lithiasis* or *pyelonephritis* in *late hyperparathyroidism*

X ray *studies to demonstrate tumors of the adrenal glands* have been used for several years *Intravenous urograms* may demonstrate a downward displacement of one kidney by such a tumor or an occasional deformity of the upper calyx because of pressure from above Occasionally *retrograde pyelograms* will demonstrate these changes more clearly In each instance the roentgenologist should be advised of the reason for the study so that films will be taken to include the entire adrenal region Occasionally *the tumor will produce a definite soft tissue shadow* which however must not be confused with the outline of the fundus of the stomach or a lesion in the tail of the pancreas However some good sized adrenal tumors do not markedly displace the kidney and may be anterior or posterior to it The writer recalls one adrenal tumor half the size of a football which was not located definitely as to either right or left side by x ray studies (including pyelograms) before operation *Contrast studies* have been used for several years *as a further step in investigating the* adrenal area The introduction of air or gas into the perirenal space has been utilized and is still being used in some clinics However it is a dangerous procedure and has

occasionally resulted in sudden deaths This tragic accident has occurred in our experience and has resulted in the abandonment of this procedure by the writer and the con

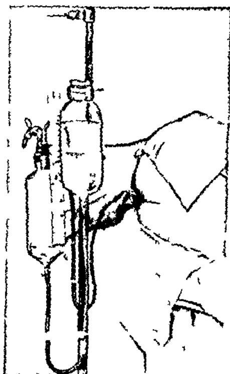


FIGURE 7 Apparatus for injection of oxygen into retroperitoneal space for extraperitoneal pneumography Note that the oxygen is injected under pressure of 20 cm of water (Courtesy of Dr Donald R Smith)

clusion that surgical exploration of the abdomen is safer Since the procedure is still used in some centers it is only fair to admit that refinements of technique or the use of gases other than air (oxygen carbon monoxide carbon dioxide) may increase the safety factor

More recently the *introduction of gas retroperitoneally*

has been suggested by Rivas[4] and further reported on by Steinbach, Lyon, Miller and Smith.[5] This has come to be known as *"extraperitoneal pneumography"* and is accomplished as follows.

Method. The patient is placed on the right side and the skin between the tip of the coccyx and the rectum is infiltrated with 1% novocaine. Anesthetic ointment is applied to the anus. After allowing time for the anesthesia to take effect, a #19 spinal needle is inserted 1 or 2 cm. below the tip of the coccyx in the midline. An index finger is placed in the anus to guide the needle which is introduced close to the sacrococcygeum in order to avoid puncturing the rectum. The tip of the needle should reach the retroperitoneal tissues 1 to 2 cm., above the tip of the coccyx. After aspiration with a syringe to be sure that the point is not in a blood vessel, the apparatus for delivery of oxygen is attached by sterile tubing with a cotton filter. A two-bottle system is used that previously has been filled with 1000 to 1200 cc. of oxygen. By adjusting the levels so that a pressure of 20 cm. of water is exerted, the gas will flow in without any great discomfort. A total amount of 15 cc. of oxygen per kg. of body weight is usually satisfactory. If this pressure does not cause the gas to enter the retroperitoneal space, the position of the needle should be changed. The oxygen tends to seek the highest level, and one half of the oxygen is given with the patient on the right side. A tympanitic note on percussion of the flank reveals that the gas has reached that area. Then the patient is turned to the left side and the second half of the oxygen is injected without moving the needle—this ascends to the other flank. The patient is then left prone until films are taken, at which time the head of the table is raised 15°. Roentgenograms can be taken at once, and if not satisfactory the position of the patient can be changed as indicated and the films repeated within one or two hours. Stereoscopic anteroposterior and lateral views of the ab-

occasionally resulted in sudden deaths This tragic accident has occurred in our experience and has resulted in the abandonment of this procedure by the writer and the con

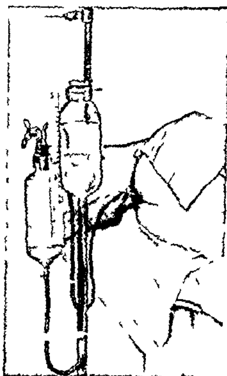


FIGURE 7 Apparatus for injection of oxygen into retroperitoneal space for extraperitoneal pneumography Note that the oxygen is injected under pressure of 20 cm of water (Courtesy of Dr Donald R Smith)

clusion that surgical exploration of the abdomen is safer Since the procedure is still used in some centers it is only fair to admit that refinements of technique or the use of gases other than air (oxygen carbon monoxide carbon dioxide) may increase the safety factor

More recently the *introduction of gas retroperitoneally*

*Arteriography* offers another method for identification of adrenal tumors by x ray The translumbar approach can be used and is performed as follows [6 7]

Method After preparation of the patient with enemas and sedation a scout x ray film of the lower thorax is taken A general anesthetic is then administered, usually pentothal supplemented by intratracheal gas and oxygen A 17 gauge 6 inch needle is then introduced just beneath the twelfth rib posteriorly about 8 cm to the left of the spinous process It is directed medially and cephalad along the body of the 12th thoracic vertebra to enter the aorta Once the needle is in place the injection is begun immediately using 12 cc of contrast medium (70% diodrast—patient previously tested for sensitivity) Pressure for the injection can be manual from a syringe [6] or from an injection apparatus to give greater pressure [7] (Two 18 gauge needles have been used by some workers allowing *simultaneous injection of the contrast medium* by two operators)

The first x ray film is taken as the last cc. of the material is injected and a second as soon as the cassette can be changed [6] However if a mechanical cassette changer is available multiple films can be exposed in a few seconds [7]

The renal circulation usually is well demonstrated and any extension to an adrenal tumor would be seen Also any variations in origin of the adrenal circulation may be noted

This method is still in the stage of development but shows promise of occasional usefulness when other simpler measures have failed to demonstrate a suspected adrenal tumor

## OTHER X RAY STUDIES

*Films of the hands* and also of the *feet* may demonstrate early changes in acromegaly *Lateral tufting of the terminal*

dominal and adrenal region are usually taken and tomography may be helpful if any confusion is caused by gas in the stomach or intestinal tract Satisfactory films are obtained up to 3 hours after the oxygen is introduced but after that length of time absorption begins and at 18



FIGURE 8 X ray film following extraperitoneal pneumogram Shows tumor in right adrenal area which was proved by operation to be a pheochromocytoma (Courtesy of Dr Donald R Smith)

hours only about 30% remains In six days only a trace can be found Experience with this has been so satisfactory that it is now frequently performed as a come and go procedure without hospitalizing the patient The gas behind the adrenal usually furnishes a satisfactory contrast so that the gland can be outlined definitely

*syndrome* and may be so pronounced that vertebral col lapse occurs causing the typical kyphosis Osteoporosis may also be seen in *senile* or *post menopausal states* and occa sionally in *hyperthyroidism hyperparathyroidism* or *ovarian aplasia*

X rays in advanced *hyperparathyroidism* also may show characteristic sub periosteal cysts which can be widespread This *osteitis fibrosa cystica* originally described by von Recklinghausen may result in spontaneous fractures or even softening and bending of the skeleton Occasionally disseminated osteitis fibrosa cystica may be encountered as part of the clinical picture of *polyostotic fibrous dysplasia* This rare condition more recently called Albright s dis ease[8] is characterized by the above bone changes with a segmental distribution plus areas of cutaneous pigmenta tion in somewhat related distribution and also sexual and somatic precocity when it occurs in the female

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*phalanges* giving a cauliflower like appearance to the phalangeal tips is a pathognomonic sign of acromegaly and may be helpful in the early diagnosis of the disease Individuals who have done hard work with their hands may have widening suggesting this lateral tufting but not to

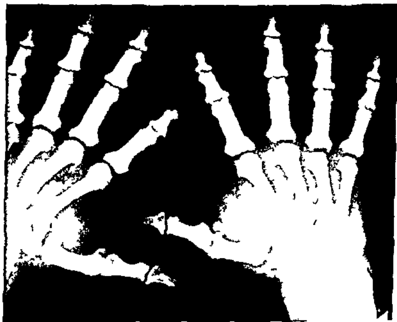


FIGURE 9 X ray study of hands in acromegaly Note characteristic cauliflower like appearance of terminal phalanges caused by lateral tufting There is also some general widening of the bones (After R F Escamilla Diseases of the Pituitary in Musser Wohl *Textbook of Internal Medicine* 5th Edition Philadelphia Lea and Febiger 1951)

the degree seen in acromegaly The changes in the toes are similar though not as marked

Among changes in the skeleton generally *osteoporosis* is occasionally important although not limited to endocrine disease This is a characteristic finding in *Cushing s*

*syndrome* and may be so pronounced that vertebral col lapse occurs causing the typical kyphosis Osteoporosis may also be seen in *senile* or *post menopausal states* and occa sionally in *hyperthyroidism hyperparathyroidism,* or *ovarian aplasia*

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*Chapter VII*

# URINE HORMONE TESTS

THE FIELD of measurement of hormones or their excretion products in the urine has been one of considerable interest and activity in recent years Such investigations have helped to clarify the pathologic physiology in some of the endocrine syndromes Occasionally one of the tests is of critical importance in diagnosis Many are complicated biological assays and are not available except in research centers However increasing interest has resulted gradually in greater availability of several of the tests Those most generally in use at present are

1 The urine pregnancy tests
2 Measurement of 17 ketosteroids
3 Measurement of pituitary gonadotropins (follicle stimulating hormone—FSH)
4 Measurement of pregnandiol excretion

In addition to the above tests for excretion of *corticoids* from the adrenal cortex may be helpful clinically and are becoming more widely available These include the measurement of the *11 oxysteroids*—a chemical test which can be performed by the copper reducing method of Talbot and co workers[1] or the formaldehyde producing method of Daughaday Jaffe and Williams[2] A biological test is also available as described by Venning Kazmin and Bell[3] which measures the *cortical hormones having a glycogenic action* Adrenalectomized mice are used for this test and the factor measured is the amount of glucose taken up by the liver as a result of the glycogenic activity of the unknown A more recent test for corticoids using the phenyl

hydrazine color reaction has been described by Porter and Silber[4] This has been applied to the measurement of *urinary compound E and F* output by Carroll *et al*[5] using a chloroform extraction and by Thorn[6] using a butanol extraction

*The 11 oxysteroids glyconic corticoids and compound E and F steroids* all show *increased excretion* in *Cushing s syndrome*

Another test for an adrenal cortical steroid in the *Allen test*[7] for dehydroisoandrosterone This has been reported to be of particular value in *differentiation of adrenal cortical tumor from hyperplasia* being positive in the presence of tumor and negative with hyperplasia A more recent method utilizing the Pettenkofer reaction has been reported by Landau and co-workers[8] All of these tests are promising and deserve more extensive trial and utilization

Another promising urine hormone test is that for the second pituitary gonadotropic hormone (the *luteinizing hormone (LH) or interstitial cell stimulating hormone (ICSH)* MacArthur[9] has reported a method based upon the effect of the extract on the weight of the ventral prostate in hypophysectomized rats

Measurement of *urine estrogens* is occasionally performed but an entirely satisfactory method is not yet available Two types of tests are used The first is a biological test using the effect on the vaginal smear of castrated rats as an end point as reported by Palmer[10] The second is the fluorometric test as suggested by Jailer[11] and modified by Engel *et al*[1]

## URINE PREGNANCY TESTS

The classical *Ascheim Zondek* test was the earliest of this group of tests which are dependent upon the increased excretion of chorionic gonadotropin producing the charac

teristic reaction Originally immature white female mice were used and the urine injected twice daily for three days At 96 hours the mice were autopsied and the ovaries examined for the hemorrhagic follicles which indicated a positive result A later modification used immature female rats After a single urine injection the maturity of the vaginal smear indicated the positive reaction [13] However this also required 96 hours for completion

*The Friedman test* was found to give a more rapid answer with comparable reliability and has been widely used An adult female rabbit is used which has been isolated from the male for at least three weeks Ten cc of urine to be tested is administered intravenously in the ear vein and repeated in 24 hours In 48 hours the rabbit is sacrificed and the ovaries examined The presence of macroscopic hemorrhagic follicles indicates a positive test

*The Galli Mainini test* using the male South American toad is the most recent and most rapid test available The *male North American frog* (Rana pipiens) has also been found to be a satisfactory test animal as reported by Robbins and Parker [14]

Method The patient is advised not to drink any fluid after lunch of the day before The morning urine specimen is collected the next day and used for the test Five cc of filtered urine is injected subcutaneously into the dorsal lymph sac of each of two male frogs A drop of urine is taken from the cloacal openings of the frogs by pipette one and two hours later and examined microscopically as a hanging drop preparation in reduced light If spermatozoa are present the test is positive as the increased amount of the chorionic gonadotropin in the patient s urine has caused a seminal discharge

This test will undoubtedly become increasingly popular since frogs are inexpensive easy to maintain need not be

sacrificed and can be used repeatedly if rested for about one week between tests The result is quickly available (in two hours) and is 96 to 98% reliable

Other tests have been advocated but are not in general use These include that using the female *South African clawed frog* (Shapiro and Zwarenstein) with the positive reaction being the extrusion of ova —and the *Guterman test*[11,12] —a colorimetric test depending upon increased urinary excretion of pregnandiol with pregnancy

In the *male*, a testicular *chorionepithelioma* will cause a *positive pregnancy test* because of the increased excretion of gonadotropins

## 17 KETOSTEROIDS

These are excretion products of hormones originating in the testes of the male and in the adrenal cortices of both sexes They are considered to be an index though not a true measure of androgenic function in the individual being tested The test is a chemical one with measurable colorimetric end point as a result of the Zimmermann reaction and has added considerably to our knowledge of testicular and adrenal function in certain endocrine diseases Accumulated experience has shown that the results parallel the androgen excretion when measured biologically by the effect on the size of the comb of a capon Even though all of the 17 ketosteroids are not androgens and all androgens are not 17 ketosteroids the test is helpful and is assuming an important place in the practice of clinical endocrinology

**Method.** That in use at present in the Metabolic Laboratory of the University of California Hospital is a modification of that described by Holtorff and Koch.[15]

A 24 hour urine sample is collected with the jug used

teristic reaction Originally immature white female mice were used and the urine injected twice daily for three days At 96 hours the mice were autopsied and the ovaries examined for the hemorrhagic follicles which indicated a positive result A later modification used immature female rats After a single urine injection the maturity of the vaginal smear indicated the positive reaction[13] However this also required 96 hours for completion

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This test will undoubtedly become increasingly popular since frogs are inexpensive easy to maintain need not be

pump. Any remaining water can be evaporated slowly at relatively low temperature. The cooled flask may be placed in a desiccator overnight and the dried extract can be stored indefinitely.

*Determination by Zimmermann reaction* is accomplished as suggested by Callow, Callow, and Emmens[14] and Holtorff and Koch[15] with some modification. The dried extract is dissolved in 2 cc. of absolute alcohol, heating in a pan of hot water at 50 C. to aid solution if necessary. 0.2 cc. of this extract is used for the reaction. Klett tubes are set up as follows:

| | |
|---|---|
| *a—Unknown—* | 0.2 cc. of the urine extract |
| | 0.2 cc. metadinitrobenzene solution (prepared fresh daily by dissolving 34.5 mg. of purified metadinitrobenzene crystals[*] in 3 cc. of absolute alcohol) |
| | 0.2 cc. 5 N aqueous potassium hydroxide |
| *b—Pigment Blank—* | 0.2 cc. absolute alcohol |
| | 0.2 cc. alcoholic extract of urine |
| | 0.2 cc. potassium hydroxide (as above) |
| *c—Standard—* | 0.2 cc. dehydroisoandrosterone solution (prepared by dissolving 25 mg. of dehydroisoandrosterone in 100 cc. of absolute alcohol—keeps relatively well in refrigerator) |
| | 0.2 cc. metadinitrobenzene solution (as above) |
| | 0.2 cc. potassium hydroxide (as above) |
| *d—Reagent Blank—* | 0.2 cc. absolute alcohol |
| | 0.2 cc. metadinitrobenzene solution (as above) |
| | 0.2 cc. potassium hydroxide (as above) |
| *e—Klett Blank—* | 0.4 cc. absolute alcohol |
| | 0.2 cc. potassium hydroxide (as above) |

All tubes are then stoppered, shaken, and placed in the dark in a constant temperature water bath at 25° C. for 105 minutes. This causes the Zimmermann reaction producing a characteristic red color which can be measured quantitatively. Immediately thereafter 8 cc. of 80% alcohol is added to each tube, diluting in the order in which the tubes are to be read. They are mixed by inversion and read on the Klett-Summerson photoelectric colorimeter within 10 minutes using a 540 filter.

containing 15 cc of concentrated hydrochloric acid or 5 cc. of 50% sulfuric acid as preservative (Five cc 2% thymol in glacial acetic acid can also be used and if the specimen is kept refrigerated not only the 17 ketosteroids but also the compound L and F content can be run from the same urine collection) On arrival at the laboratory the total volume is measured and a 50 cc aliquot is taken for the test *Hydrolysis* is accomplished with some modification of the method suggested by Drekter *et al*[16] and Vestergaard[17] by acidifying the sample with 10 cc of concentrated hydrochloric acid in a 125 cc Erlenmeyer flask with ground glass stopper This is placed in a constant temperature water bath at 60 C for 15 minutes and then cooled After cooling the sample can be kept in a refrigerator for 24 hours at this stage

*Extraction* is performed by a modification of the methods of Drekter *et al*[16] and Holtorff and Koch[15] The cooled sample is poured into a 250 cc separatory funnel and mixed twice with 20 cc portions of ether and once with a 10 cc portion shaking 1½ minutes each time (a pinch of detergent such as Vel or Dreft may aid the separation of ether layers—not necessary routinely)

*Washing* is accomplished by combining the 3 ether extracts in a 250 cc separatory funnel and adding 10 cc portions of a freshly made sodium hydroxide sodium hydrosulfite solution as suggested by Talbot *et al*[18] This is freshly prepared by adding 10 gm of sodium hydrosulfite powder to 100 cc of 10% sodium hydroxide The washing is performed 3 times shaking 1 minute each time This removes the phenolic fraction which contains estrone (also a 17 ketosteroid) The extract is then washed 3 times with distilled water or until the washing is neutral

*Evaporation* is the next step and for this the ether extract is transferred to a 50 cc Erlenmeyer flask and evaporated to dryness over a hot plate using grooved corks Reduced pressure for this is obtained by using a water

pump Any remaining water can be evaporated slowly at relatively low temperature The cooled flask may be placed in a desiccator overnight and the dried extract can be stored indefinitely

*Determination by Zimmermann reaction* is accomplished as suggested by Callow Callow and Emmens[19] and Holtorff and Koch[15] with some modification The dried extract is dissolved in 2 cc of absolute alcohol heating in a pan of hot water at 50 C to aid solution if necessary 0 2 cc of this extract is used for the reaction Klett tubes are set up as follows

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| | 0.2 cc metadinitrobenzene solution (prepared fresh daily by dissolving 34 5 mg of purified metadinitrobenzene crystals[20] in 3 cc of absolute alcohol) |
| | 0 2 cc 5 N aqueous potassium hydroxide |
| *b—Pigment Blank—* | 0 2 cc absolute alcohol |
| | 0.2 cc alcoholic extract of urine |
| | 0 2 cc potassium hydroxide (as above) |
| *c—Standard—* | 0 2 cc. dehydroisoandrosterone solution (prepared by dissolving 25 mg of dehydroisoandrosterone in 100 cc of absolute alcohol—keeps relatively well in refrigerator) |
| | 0 2 cc metadinitrobenzene solution (as above) |
| | 0 2 cc. potassium hydroxide (as above) |
| *d—Reagent Blank—* | 0 2 cc absolute alcohol |
| | 0 2 cc metadinitrobenzene solution (as above) |
| | 0 2 cc potassium hydroxide (as above) |
| *e—Klett Blank—* | 0 4 cc. absolute alcohol |
| | 0.2 cc potassium hydroxide (as above) |

All tubes are then stoppered shaken and placed in the dark in a constant temperature water bath at 25° C for 105 minutes This causes the Zimmermann reaction producing a characteristic red color which can be measured quantitatively Immediately thereafter 8 cc of 80% alcohol is added to each tube diluting in the order in which the tubes are to be read They are mixed by inversion and read on the Klett Summerson photoelectric colorimeter within 10 minutes using a 540 filter

The following calculation gives the result

17 ketosteroids in mg per 24 hours =

$$\frac{\text{corrected reading of unknown}}{\text{corrected reading of standard}} \times \frac{\text{24 hour volume}}{100}$$

The *normal range* for adults is considered to be *males* 10 to 20 mg average 15 mg per 24 hours and *females*

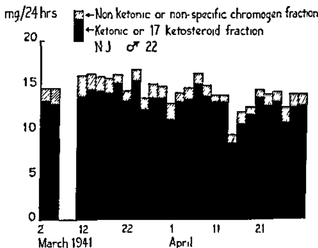



FIGURE 10 Graph demonstrating variation in 17 Ketosteroid output of a normal man over a period of 2 months 48 hour urines were tested Separation into ketonic and non ketonic fractions also indicated (Courtesy of Dr Sidney C Werner *J Clin Endocrinol 1* 952 Dec 1941 and *Bull Univ Calif Med Center 2* 399 Feb 1951)

5 to 15 mg average 10 mg per 24 hours In *children* the values are near 0 until the age of 8 By 12 they are 1 5 to 5 mg and then increase gradually to adult levels at about the age of 16 No marked sex difference is apparent until this time There is also moderate day to day variation in excretion as demonstrated by Werner[21] (see Figure 10)

* Corrected reading is colorimeter reading corrected for pigment and reagent blanks

thus rendering meaningless the reporting of results to several decimal places and diminishing the significance of slightly high and slightly low levels

Also the result expressed above represents total neutral 17 ketosteroids—the end point for most clinical tests Further fractionation is possible with separation into ketonic and non ketonic fractions using Girard s reagent T The non ketonic is usually 10 to 15% of the total neutral though occasionally it may be 25% This separation has not added to the clinical usefulness of the test and is suggestive at best However the ketonic fraction may be further separated into alpha and beta fractions with digitonin The *beta fraction* is usually under 5% but may be increased under certain conditions and *if increased to over 50% with a total excretion of over 50 mg* per day is considered to be evidence for the presence of an *adrenal cortical carcinoma* The simpler *Allen test*[7] (page 67) is also reported to be positive in malignant adrenal tumors

Studies of 17 ketosteroid urinary excretion in various endocrine diseases have shown the following *low levels* are seen in *hypophyseal infantilism Simmonds disease Addison s disease* (especially in women where excretion should be close to O ) *myxedema* and occasionally in *anorexia nervosa* The last is unfortunate as it was orginally hoped that this test would be of critical importance in differentiating Simmonds disease and anorexia nervosa While it is true that values in Simmonds disease are generally lower than those in anorexia nervosa there is overlapping of the ranges in the two conditions so that in an individual patient the test may be helpful but not decisive

*High 17 ketosteroids* are found with *adrenal cortical carcinoma* or *malignant adenoma* and *moderately high*

figures are found in *adrenal cortical hyperplasia*, especially with the *adrenogenital syndrome* Excretion in patients with carcinoma is usually over 50 mg per day and may be as high as 1,000 mg (1 gm) plus per day As already stated if the *excretion is over 50 mg* per day and if further fractionation shows that *over 50% of this is the beta fraction a tentative diagnosis* of *adrenal cortical carcinoma* seems justified In *adrenal cortical hyperplasia*, excretion of from normal to 100 mg per day is seen The *Allen test* (p 67) *may also help to differentiate between adrenal carcinoma* and *hyperplasia* Occasional instances of verified adrenal cortical carcinoma have been reported in which the 17 ketosteroid excretion has been normal In spite of this the test has its greatest clinical importance in this condition

An occasional patient with adrenal cortical carcinoma will be found among the many women complaining of increased hirsutism (heterosexual hypertrichosis) and this test should be run routinely in such patients This has been emphasized in a recent report by Kinsell and Lisser [3] of a patient with hirsutism of 14 years duration but without other evidences of defeminization or masculinization such as amenorrhea infertility atrophy of the breasts deepening of the voice or enlargement of the clitoris A 17 ketosteroid test done as a matter of routine interest showed a surprisingly high excretion of 82 mg per 24 hours of which 55% was *the beta fraction Further investigation* revealed an adrenal cortical tumor approximately four inches in diameter This was removed surgically and proved to be a malignant adenoma *In such a* patient the tumor probably would not have been suspected *had the 17 ketosteroid excretion not been measured* Therefore the test with all of its imperfections had critical clinical value

*Slightly low levels* of excretion are found in *male eunuchoidism male castrates burned out gigantism and acromegaly, diabetes mellitus* in the *male climacteric* and in chronic *debilitating diseases* The level tends to fall in *old age*

*Slightly high levels* are found in *Cushing s syndrome,* simple *hirsutism* (occasionally to 25 to 30 mg per day) *active acromegaly and gigantism arrhenoblastoma precocious puberty pregnancy* and in *hyperthecosis*

However the variation of daily excretion in the same individual (as illustrated in Figure 10) and the occasional variation from laboratory to laboratory depreciates the clinical value of these *slightly high* and *slightly low* levels Indeed these minor changes are by no means invariable in the diseases mentioned

## FOLLICLE-STIMULATING HORMONE EXCRETION (FSH)

This test also is occasionally helpful clinically The measurement of this pituitary gonadotropin (also called Prolan A thylakentrin or pituitary gonadotropin A) gives some indication of the level of anterior pituitary function Clinically it may aid in arriving at the proper diagnosis or the correct treatment in an individual patient The test is a biological one and requires a colony of either rats or mice depending upon the method used

The *method using rats* has been advocated by Jungck Maddock and Heller and is in use in several centers In this the urine concentrate is prepared by ultrafiltration and then is injected six times into female rats at 12 hour intervals Twenty four hours after the last injection the uterus and ovaries are removed and weighed—the weights being recorded as end points

However the *method using mice* and recording the

results in mouse units is in more general use at present and is the one used in the Endocrine Laboratory of the Division of Obstetrics and Gynecology at the University of California Hospital It was originally described by Zondek[25] and modified by Klinefelter Albright and Griswold[26]

Method The present method used is a modification of that of Dekanski[27] and Bradbury Brown and Brown[28] A 24 hour urine specimen is collected and kept under refrigeration After measuring if there is any toluene preservative present it is filtered The pH is then adjusted to 4 0 with a Beckman pH meter using 20% hydrochloric acid Fifteen cc of 20% kaolin in water is then added and after thorough stirring is allowed to settle for 1 hour The supernatant fluid is removed by suction and discarded The remainder is transferred to a centrifuge tube and centrifuged for 10 minutes at moderate speed The supernatant fluid is then decanted and 10 cc of 0 1N sodium hydroxide are added to the kaolin with thorough mixing The mixture is again centrifuged for 10 minutes and the supernatant transferred to a small beaker Using 5% hydrochloric acid this is brought to pH of approximately 7 as determined by nitrazine paper It is then made up to a volume of 12 5 cc with distilled water and placed in a tube to be kept in the deep freeze until ready for the test

*Test* The concentrate as prepared above represents a level of five mouse units and is injected subcutaneously undiluted into two female mice 18 to 21 days of age They are given 0 5 cc twice daily for 2 days and once on the morning of the third day making the total dose 2 5 cc On the fourth day the mice are autopsied and the uterus stripped of fat blotted and weighed If the weight is above 15 mg the test is positive (the normal weight being from 3 to 8 mg)

The level to be tested must be decided in advance The

concentrate can be prepared for testing at other levels by dilution with distilled water the amount being figured by dividing the mouse unit level by 5—i e

> 40 mouse units—dilution 1 8 (1 cc concentrate plus [illegible] cc distilled water)
> 80 mouse units—dilution 1 16 (1 cc concentrate plus 15 cc distilled water)

The *normal range* in adults is considered to be 5 to 50 mouse units per 24 hours It has been found satisfactory to run an unknown urine at two levels—5 and 80 mouse units If the test proves negative at both levels the excretion is below normal Should the result be positive at 5 mouse units and negative at 80 units the excretion is probably within the normal range of 5 to 50 mouse units If positive at both 5 and 80 unit levels then the excretion is above the normal range If desired later specimens can be tested at higher lower or intermediate levels as indicated by the results of the original test

Thus the test is moderately complicated and requires an animal colony—but is occasionally of sufficient usefulness and importance to justify the effort of setting it up It is of crucial importance in the differentiation between *hypophyseal infantilism (pituitary dwarfism) (low excretion)* and *ovarian aplasia (high excretion)* In the latter condition it is important that the patient not receive any estrogenic therapy for about 1 month before the test as estrogens depress the high levels (occasionally 300 to 400 mouse units) to normal In addition to *ovarian aplasia high levels* of excretion are found in the *climacteric* in both sexes This finding is more frequent in females but normal levels may be found Thus it is occasionally helpful in diagnosis of the climacteric but unfortunately is not an invariable finding *Eunuchs castrates* and certain types of *eunuchoidism* also show *increased FSH excretion* as do patients with *Klinefelter s syndrome* The latter syndrome

was described by Klinefelter Reifenstein and Albright[29] in 1942 It occurs in males and is characterized by gynecomastia small testes with azoospermia and increased excretion of FSH On testicular biopsy the tubules are found to be hyalinized but the interstitial cells retain normal appearance

*Low levels* of FSH excretion are found in *hypopituitarism* including *hypophyseal infantilism hypogonadotropic eunuchoidism* and *Simmonds disease* Unfortunately low levels are occasionally found also in *anorexia nervosa,* so once again in the individual patient the test may not be of crucial differential importance

A summary of experiences with this test in various diseases was published by the writer in 1949

## PREGNANDIOL EXCRETION

This is an excretion product of progesterone and has been studied considerably in centers where there is active investigation in the field of obstetrics and gynecology A *fall* in pregnandiol excretion during pregnancy has been considered an indication of *threatened abortion* The method of Venning[30] measures the excretion of sodium pregnandiol glucuronidate In this the urine is extracted with butyl alcohol and the residue treated so that it can be weighed Use of a conversion factor (0 597) gives the amount of pregnandiol

The method of Guterman[31,3] is a colorimetric one and is considered of sufficient accuracy to be used as a test for pregnancy

In *habitual abortion* the *lowered excretion* of pregnandiol may indicate the need for prophylactic use of progesterone and may be a guide to proper dosage An *increased output* has been reported with *polycystic ovaries* (Stein Leventhal syndrome) by Fischer and Riley[33]

Despite the above indications the test is not as widely used as in the past It is possible that the clinical help derived has not been sufficient

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*Chapter VIII*

# VAGINAL SMEAR—CERVICAL MUCUS

EXAMINATION of the vaginal smear is simple and easily accomplished as an office procedure It is of consider able assistance to the clinician in estimating the level of ovarian function and also can be used as a guide in deter mining the adequacy of therapy with estrogens

The cells collected for examination are those being desquamated from the vaginal epithelium and the upper genital tract Consideration of a few fundamental facts will indicate the basis for interpretation

Estrogens cause an increase in the layers of cells in the *vaginal epithelium If a large amount of estrogen is pres ent* there are many rows of epithelial cells the most superficial of which are squamous flattened and cornified If the level of estrogen falls the number of layers dimin ishes and the flattened cornified cells tend to disappear with precornified cells of the intermediate layers predomi nating These are moderately flat but not the true squa mous type If the estrogen level is low the epithelium is thinner and cells of the basal layer appear These are small of round or oval shape with relatively large nuclei The *nuclei of the deeper cells are distinctly hyperchromatic* The picture seen in smears varies from the extreme hy poestrin picture of the atrophic postmenopausal state to that seen during pregnancy when the system is surfeited with estrogens However the change is sensitive enough to indicate the differences in estrogen level during the normal menstrual cycle

Method The smear must be taken before the usual

vaginal examination with lubricant is performed and at least 24 hours after the last douche A slightly curved glass pipette six inches (15 cm) in length and 0 5 cm in diameter is used At one end is a rounded tip with relatively small opening and at the other is attached a rubber bulb of one to two ounce capacity The bulb is compressed and the tube inserted deep to the posterior fornix of the vagina and turned from side to side The suction created by the bulb is allowed to take up the vaginal secretion The tube is withdrawn and the contents expelled onto a clean glass slide spreading the secretion and fixing *immediately* for drying causes distortion of the cell picture Swabbing or scraping of the mucous surfaces has been recommended but is generally thought to distort the cells as well as to cause false exfoliation However if the vaginal contents are very atrophic and dry this procedure may be necessary (the fact that fluid is unobtainable is an important indication of hypoestrinism)

The *fixative* used is made up of equal parts of 95% ethyl alcohol and ether and should be in a Coplin jar or receptacle of similar size and shape beside the examining table for immediate use A paper clip on each glass slide is sufficient to keep the slides separated in the solution Fixation is accomplished in a few minutes but is more certain if continued for one hour The slides can be left in this solution without harm for one week

*Staining*—The Shorr Trichrome[1] stain is satisfactory for office use It can be made up as follows

| | | |
|---|---|---|
| Biebrich Scarlet (aqueous solution) | 0 5 | gm |
| Orange G | 0 25 | gm |
| Fast Green FCF | 0 075 | gm |
| Aniline Blue (aqueous solution) | 0 04 | gm |
| Phosphotungstic Acid | 0 | mg |
| Phosphomolybdic Acid | 0 5 | gm |
| Glacial Acetic Acid | 1 0 | cc. |
| All dissolved in 50% ethyl alcohol | 100 | cc |

This stain is applied to the slide by dropper or in Cop-

lin jar and allowed to stand for 1 minute Then the slide is dipped 10 times in 70% alcohol and 10 times in 95% alcohol well cleaned with xylol and mounted The fully cornified cells in addition to their typical shape with small nuclei stain a reddish orange while the cells from the deeper layers stain green or blue A differential count can be made counting 100 to 300 cells and reported as the percentage of acidophilic cornified cells in the smear

**Normal variations** These are seen during the normal menstrual cycle as follows

**Menstrual phase** Many red cells are present The vaginal cells chiefly folded thickened and mucified superficial layer cells stain poorly Dark pigment like granules may be present After a day or two more basophilic cells appear

**Post menstrual and early follicular phase** The estrogen level is rising and cells of the intermediate level appear in increasing numbers Cornified elements increase gradually (up to 30%) and are more or less separate with folded cyto plasm

**Pre ovulatory phase** This lasts for 24 to 48 hours and is characterized physiologically by a rapid rise in estrogen production The vaginal smear reflects this with a rise in the cornified elements to 40 to 50% Doderlein s bacilli are in evidence

**Ovulatory phase** This lasts only about 24 hours and shows the intermediate peak of cornification with cornified elements at levels of from 50 to 75% and very few leuco cytes The vaginal cells stain brightly many occur in shingle like plaques and the nuclei are pyknotic and may be fragmented

**Post-ovulatory phase** At this time there is a fall in estro gen production for two to three days Progesterone effect also appears and opposes somewhat the cornifying effect of the estrogens The cornified cells begin to show folding

# CERVICAL CELL PATHOLOGY
## IN SQUAMOUS TISSUE

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### GRADES AND CELL TYPES IN "SURFACE BIOPSIES" OF CELLS

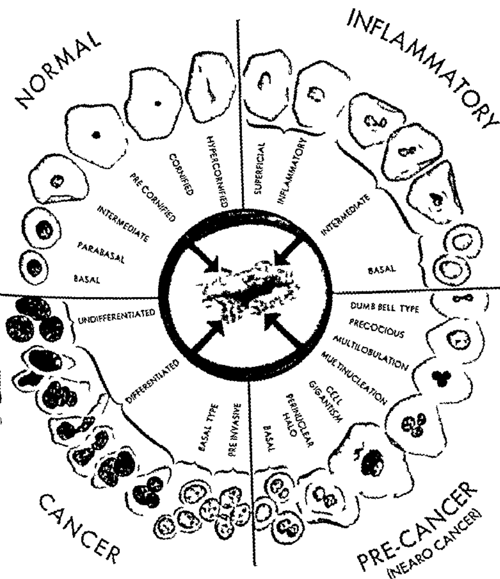



FIGURE 11 Cell types seen in vaginal smears (After *Ciba Clinical Symposia* Vol 3 #4 June 1951 Courtesy of Dr E Oppenheimer and Dr J E Ayre *Cancer Cytology of the Uterus* New York Grune and Stratton 1951)

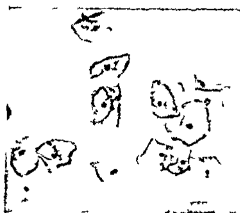


FIGURE 12 Vaginal smear taken in preovulatory phase (Shorr stain) Note high percentage of cornified cells (After *Ciba Clinical Symposia* Vol *1* #4 p 107 April May 1949 courtesy of Dr R Greenblatt and Dr E Oppenheimer)

and curling of the edges and the proportion of basophilic cells increases Percentage of acidophilic cornified cells is usually about 50% Leucocytes and more densely staining mucus also appear in the smear

**Luteal phase** This lasts for eight to 10 days during which the progestational effects are more marked The acidophilic cornified cells become more curled and folded and the outlines may be irregular There is some tendency to clumping The nuclei remain pyknotic Percentage of acidophilic cornified cells gradually drops from 50% to the 20 to 30% level The leucocytes increase further and the basophilic staining of the mucus is more prominent Mixed bacterial flora is seen with cocci predominating

**Pre menstrual phase** This is caused by the drop in levels of both estrogen and progesterone one to five days before the onset of menstrual flow The percentage of cornified cells drops rapidly to 20% or under and those remaining are folded irregular and stain poorly Leucocytes and mucus are abundant

## ESTROGEN DEFICIENCY

Varying degrees cause different findings in the smear as follows

**Marked deficiency** (seen in *castrates* and *senile women* —occasionally with *severe menopause syndrome* and with *androgen therapy*) Nearly all the cells are from the basal layer and the percentage of cornification is very low Mitotic activity may be seen in the basal cells Leucocytes are numerous with many bacilli and cocci and may be excessive with associated atrophic vaginitis

**Moderate deficiency** (most common in the *menopause* or *post menopausal states*—also with *long standing ovarian deficiencies in younger women*) Most of the cells are from the basal layer but some intermediate layer cells are seen

Occasional basophilic squamous cells are noted but the percentage of acidophilic cornified cells is low

Leucocytes are present and a moderate amount of mucus is in evidence

Slight deficiency (seen in *mild menopause syndrome* or in *younger women* with *moderate ovarian deficiency*) Most of the cells are from the intermediate layer with some cells from the basal layer Cornified cells when counted are usually at levels of 20% or under A moderate number of leucocytes and some mucus are present

## PREGNANCY

In this condition the findings are the result of progressive increase of estrogens and progesterone After two to four weeks the number of acidophilic cornified cells decreases and the smear shows increasing numbers of folded basophilic cells and midzonal cells which are grouped and show accentuation of *the margins* with curling of the navicular shapes *If abortion is threatened* red cells are seen and if incomplete leucocytes and histiocytes appear often in clusters or rosettes Also evidences of infection and thick strands of ropy mucus are seen

## HYPERESTRINISM

*This condition is seen with granulosa* or *theca cell tumors* of the ovary and with the administration of *large doses of estrogens* The smear shows a high percentage (up to 90%) of the acidophilic cornified cells These cells may be small and the nuclei fragmented or even absent Leucocytes are rare and the mucus though abundant stains poorly

An important *confusing factor* in the interpretation of vaginal smears is the presence of *infection,* particularly

cervicitis or vaginitis. Some cornification may result with *trichomoniasis* which is not due to estrogen effect. Also basal layer cells may appear *from ulcerated areas* when they would not otherwise be present. The presence of an unusually large number of leucocytes or of trichomonas should alert the observer to the possibility of these effects.

## DETECTION OF CANCER

The Shorr polychrome stain as described above is satisfactory for determining the estrogen level of the patient and also for following the results of estrogenic therapy. However, if the smear is to be used for the *detection of cancer cells*—the *Papanicolaou stain* is preferable. It takes somewhat longer but has the advantages of greater penetration and differential staining of thick mucoid or bloody smears.[2]

Method. After fixing in 95% ethyl alcohol and ether (equal parts) the smear is run through 70% and 50% alcohols and then stained in hematoxylin for 5 minutes. It is rinsed in distilled water followed by 0.5% aqueous hydrochloric acid and then washed thoroughly in tap water. Next it is run through distilled water and 50%, 70%, 80% and 95% alcohols and stained for one minute in 0.5% solution of Orange G in 95% alcohol (occasionally 0.015 gm. per 100 cc. of phosphotungstic acid is added). The smear is then washed in 2 changes of 95% alcohol and stained in trichrome solution for two minutes. This stain contains 45 cc. of 0.5% solution of Light Green S.F. Yellowish in 95% alcohol, 45 cc. of 0.5% solution of Eosin Yellowish in 95% alcohol, 10 cc. of 0.5% solution of Bismarck Brown in 95% alcohol, 0.2 gm. of acid phosphotungstic and 1 drop of a saturated aqueous solution of lithium carbonate. After staining the slide is rinsed 3 times in 95% alcohol, run through absolute alcohol and xylol, and mounted. With this stain the acidophilic cells

appear red to orange and the basophilic cells green or green blue The epithelial cells and erythrocytes though stained are relatively transparent

The *diagnosis of cancer* by this method has been found accurate in proper hands with diagnostic error of only 4% for squamous cell carcinoma as reported by Fremont Smith and Graham[3] With endometrial or fundal carcinoma the accuracy is lower but is still satisfactory in almost 80% of cases

## IODINE VAPOR STAIN[4]

This stain is simple rapid and is occasionally helpful It gives an indication of the *vaginal glycogen index*

Method A simple dried smear is placed face down over a shallow dish containing a small amount of Lugol s solution The iodine vapors which arise insensibly stain the glycogen containing cells in 2 to 3 minutes They should be examined immediately but can be stained in the same manner after 24 to 48 hours

The amount of glycogen in the cells of the smear indicates the *degree of estrogenic response* and can be classified in 4 grades Grade 1 shows complete glycopenia while grade 4 shows exclusively large flat deeply stained brown iodophilic cells singly or in clumps This latter corresponds to the smear of the normal proliferative phase

## CERVICAL MUCUS

Examination of mucus aspirated directly from the cervix is occasionally helpful in sterility studies Cohen Stein and Kaye[5] have pointed out that the *cervical mucus becomes profuse and thin at the time of greatest fertility* and they feel that this is a more accurate indication than observation of the basal body temperature curve The specimen



FIGURE 13 Group of cancer cells in a vaginal smear (Papanicolaou method) (After *Ciba Clinical Symposia* Vol *1* #1 Spring 1948 courtesy of Dr E Oppenheimer)

is obtained by inserting the tip of a glass cannula into the endocervix and aspirating mucus by means of gentle suction from a 10 cc syringe attached to the other end The mucus is blown out on a glass slide and a glass coverslip placed over it The thread formation (Spinnbarkeit) is then tested by lifting the coverslip until the thread of mucus breaks During the fertile period the coverslip can be lifted 10 to 20 cm while during the infertile period only 0 to 1 cm lift is possible before the thread breaks The mucus during the infertile period is also thick scant and difficult to aspirate Small doses of stilbestrol (0 1 to 0 2 mg daily) may improve the amount and thinness of the cervical mucus *Examination of the cervical mucus is especially valuable in selecting the optimal time for artificial insemination* Profuse clear mucus is also seen after administration of estrogens to the castrate and occasionally in patients with bilateral polycystic ovaries

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## Chapter IX

# ENDOMETRIAL BIOPSY

HISTOLOGICAL examination of a small portion of the endometrium can be accomplished as an office procedure It is more complicated than the vaginal smear and should be done by one with training in gynecologic methods since the cervix must be dilated and the uterine cavity entered to obtain fundal specimens these being the most satisfactory In proper hands it is safe and usually not uncomfortable for the patient and yields information regarding ovarian and corpus luteum function that cannot be obtained by other methods including vaginal smears and hormone estimations in blood and urine

Method The patient is placed in the lithotomy position and the position of the uterus determined Under direct speculum observation the cervix and the external os are cleansed with an antiseptic (70% alcohol is satisfactory) The anterior cervical lip is grasped by a single toothed cervix tenaculum and held steady while a suction curette is introduced in the direction of the fundus A circular stripping motion is made while exerting suction to obtain the specimen This is placed immediately in fixative solution (Bouin s solution Zenker s solution or 100% alcohol for glycogen staining) Sections are run through as with other histological or pathological specimens

No after care of the patient is necessary Usually only slight spotting occurs and there may be a few cramps

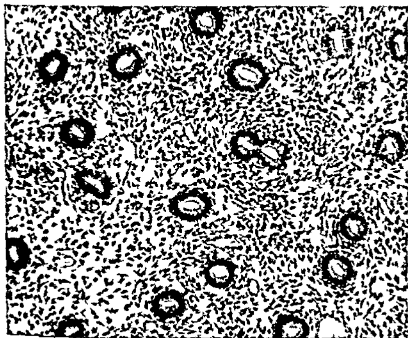


FIGURE 14 Endometrial biopsy during normal proliferative phase The glandular lumen is narrow and is surrounded by a single layer of columnar epithelium (After *Ciba Clinical Symposia* Vol *I* #5 p 138 June July 1949 courtesy of Dr E Oppenheimer)

FIGURE 15 Endometrial biopsy—early secretory phase—Note glycogen in base of cells (After *Ciba Clinical Symposia* Vol *1* #3 June July 1949 Courtesy of Dr E Oppenheimer)

The possibilities of perforation of the uterus and spread of infection with pelvic peritonitis have been pointed out but are unlikely and can easily be avoided Undue force should not be used the course of the cervix and direction of the body of the uterus should be ascertained before hand aseptic technique should be observed and patients with gonorrhea and acute cervicitis should not be sub jected to the procedure

## INTERPRETATION OF SPECIMENS

### NORMAL MENSTRUAL CYCLE

**Preovulatory phase** The estrogenic effect is dominant with typical proliferation of the endometrium which has been called the *proliferative phase* The endometrium gradually thickens and the glands increase in number and size and become more crowded and coiled The lumen di lates but the epithelium maintains a well defined even con tour Glandular epithelial cells are tall and columnar and are crowded with large dark staining nuclei in a scanty pale cytoplasm—mitoses are frequent

**Postovulatory phase** This is dominated by the effect of progesterone and secretory changes appear giving the characteristic picture of the *secretory phase* Mucus and glycogen appear in the dilated and coiled glands and the cell nuclei are pushed to a central position The glycogen vacuoles move from a basal to a peripheral position and the nuclei now round and pale migrate to the base of the cell Coiled arterioles appear with congestion and small hemorrhages in the superficial layers The cells become turgescent and pyramidal in shape and the epithelial layer becomes folded

Just before menses the glands have discharged their

secretions and leucocytes and scattered hemorrhages are prominent

With normal function an experienced observer can usually tell within a few days the time of the cycle when the biopsy was taken However in pathological conditions it may be extremely important to know exactly the *timing in the cycle when the specimen was obtained* Therefore, the dates of the biopsy and the dates of two or three preceding menstrual periods should always be noted for correlation and to aid in interpretation

For diagnostic purposes the *biopsy is best taken between the twenty first day of the cycle and the day of onset of bleeding*

With *normal estrogenic and progestational effects* the endometrium will show good development and secretory activity This is also the best evidence that can be secured that ovulation has taken place—important knowledge in *sterility studies*

## PROGESTERONE DEFICIENCY

*Moderate degree* is indicated by secretory changes less than normal in the specimen However quantitation is difficult and the possibility of a refractory endometrium must be considered

*Progesterone lack and absence of ovulation (anovulatory cycle)* This is indicated by persistence of the proliferative phase and absence of secretory changes up to the time of menstruation Glandular cystic hyperplasia may also be present due[1] to prolonged unopposed estrogenic effect

## ESTROGEN DEFICIENCY

*Complete ovarian deficiency (also seen in the menopause)*—This is indicated by a specimen of the castrate or

postmenstrual type occurring just *before* menstruation It is characterized by little or scant proliferative change and absence of secretory changes

It should be remembered that the above findings change gradually from one to the other and that there may be transitional pictures and mixed types between those absolutely characteristic Also endometrial biopsy does not help to distinguish between primary ovarian failure *(female eunuchoidism ovarian aplasia)* and that resulting secondarily from changes in function in other glands as with the *severe hypopituitarism* of *Simmonds disease*

## AMENORRHEA

Biopsies can be taken at any time and if serial studies are made at weekly or biweekly intervals there will be evidence of any cycle that is occurring including ovulation without bleeding

## HYPERESTRINISM

This is seen with *granulosa theca cell tumors* of the ovary and with *large doses of estrogens* A marked proliferative picture is seen and occasionally endometrial cystic hyperplasia is present—(the Swiss cheese endometrium of Novak) Decidua like changes have been reported with granulosa cell tumors

## EXCESS OF PROGESTERONE

This also may be present with *granulosa theca cell tumors* which have undergone luteinization—occasionally to the degree that they can be called *luteomas* It will be reflected in the biopsy specimen by varying degrees of secretory changes usually in a hyperplastic type of endometrium

secretions and leucocytes and scattered hemorrhages are prominent

With normal function an experienced observer can usually tell within a few days the time of the cycle when the biopsy was taken However in pathological conditions it may be extremely important to know exactly the *timing in the cycle when the specimen was obtained* Therefore the dates of the biopsy and the dates of two or three preceding menstrual periods should always be noted for correlation and to aid in interpretation

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## FOLLOWING ADMINISTRATION OF ANDROGENS OR ANDROGENIC TUMORS

*(Including adrenal cortical tumors and hyperplasia and arrhenoblastoma)*

Varying degrees of atrophy of the endometrium are seen With high doses of androgens it may be reduced to a thickness of only one layer of cells Ovulation will usually be suppressed so that no secretory changes are seen

## HYPOTHYROIDISM

This condition is often characterized clinically by excessive uterine bleeding In animal studies Williams Phelps and Burch[2] have demonstrated that hypothyroidism can induce glandular cystic hyperplasia Hamblen Pullen and Cuyler[3] after investigations in humans concluded that the change in hypothyroidism was a quantitative slowing down of ovarian activity rather than a qualitative effect and that such patients bled more frequently from a progestational endometrium Goldsmith Sturgis *et al*[4] have recently studied this problem and report bleeding more frequently from proliferative endometrium but also occasionally from that showing both the early and late secretory phase All abnormalities reverted to normal following adequate treatment with thyroid substance

## OTHER CONDITIONS

**Endometrial cancer** This can be diagnosed by biopsy but the incidence of positive diagnosis has been estimated only at 75%[5] Therefore the greater certainty of a dilatation and curettage with examination of the complete endometrium is preferable

*Endometrial tuberculosis* This also is occasionally encountered in this type of biopsy Typical histopathological findings of tuberculosis are seen in the specimen

temperature until delivered to the laboratory preferably within two hours The laboratory should be apprised of the time interval when the specimen is delivered Cleanliness and dryness of the bottle are important as water or other material present may radically affect the sperm

Patients usually cooperate in this manner of obtaining the specimen if the reason is explained but should they demur or deem it offensive it can be obtained by coitus interruptus This may be satisfactory but occasionally the first few drops are lost and these contain a high percentage of the spermatozoa Objections on religious grounds occasionally preclude either of these methods in which case it becomes necessary to obtain semen from the vagina as soon as possible after intercourse This method is not satisfactory as dilution with the normal acid vaginal secretion can result in a completely distorted picture It may be more satisfactory to perform a testicular biopsy Collection in a condom or sheath is usually not satisfactory as it is difficult to retrieve the full specimen and also the powder present may have a toxic effect upon the spermatozoa

## EXAMINATION OF THE SPECIMEN[1]

### 1 Gross Examination

a *Volume* Normally this is 2 5 to 5 cc with an average of 4 cc[2] Diminished volume occurs in primary or secondary male *hypogonadism* and when this is severe it may be impossible to obtain a specimen McCullagh and Schaffenburg[3] consider this a fairly good index of androgenic function with a volume under 2 cc indicating a deficiency

b *Turbidity* The semen is usually opaque especially when sperm concentration is normal If more transparent than normal decreased sperm concentration is usually present

c *Viscosity* Normally the semen is moderately viscid though by the time it arrives at the laboratory it is usually an

*Chapter X*

# SEMEN EXAMINATION

ANALYSIS of the seminal fluid is of prime importance in evaluating the spermatogenic function of the testes It also verifies patency of the tubular apparatus which conducts spermatozoa to the prostate and urethra If the seminal fluid is normal in amount and content it is safe to assume that both the spermatogenic and secretory functions of at least one testis are normal The examination is of particular value in the study of male infertility but may reveal typical changes in primary or secondary hypogonadism Some infertile men may have decreased or impaired spermatogenic function with normal secretory function so that there are no outward manifestations of endocrine disease An exception is the Klinefelter syndrome *which is characterized by other endocrine changes* especially gynecomastia

Examination of the semen is relatively simple but of course requires complete cooperation of the patient It should always be done before testicular biopsy is performed as it may eliminate the necessity for this more radical procedure

**Method of collection** The patient is advised not to have intercourse for at least five days before and not to collect a specimen during or shortly after a febrile illness It is recommended that it be obtained by masturbation and collected in a clean dry wide mouthed bottle Following collection *it should be kept at moderate room*

may vary considerably in patients with oligospermia and it is advisable to examine more than one specimen

4 Types of spermatozoa and incidence of pathological forms

For this portion of the examination a drop of semen is placed on a microscope slide and spread with another slide as in making a blood smear The smear is fixed with heat and then covered with 1% solution of chloramine for several minutes (This removes mucus and overlying spermatozoa so should not be used if very few are present) The slide is then washed with water and 95% alcohol and dried by blotting with filter paper It is then stained for two to five minutes using Ziehl Neelsen s carbol fuchsin two parts concentrated alcoholic solution of eosin one part and 95% alcohol one part Following this it is washed with water and counterstained with Loeffler s methylene blue for a few seconds It is then washed dried and examined under the oil immersion lens The heads stain a purplish color while the tails and middle portions are red Usually 300 spermatozoa are counted noting the following characteristics for classification

1 *Normal spermatozoa*—usually over 70% of the total

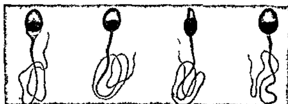


FIGURE 16 Normal spermatozoa showing head with full oval shape (After R S Hotchkiss *Infertility in Men* American Lecture Series No 53 Ed Willard O Thompson M D Springfield Ill Charles C Thomas Publisher 1952)

easy flowing liquid If watery a low sperm count may be associated while a high viscosity may impair sperm mobility

d *Chemical reaction* It is always alkaline and nitrazine paper will usually demonstrate a pH of 7 5 to 8

## 2 Motility

This should not be impaired within six hours if kept at moderate room temperature It can be observed in a drop of semen placed on a slide under a cover slip and examined with low power of the microscope A satisfactory part of the field toward the end of the cover slip is then brought under high power and 200 cells counted noting percentage of absolute nonmotility This is occasionally repeated at one two four eight and 24 hours Normally 70 to 80% of the sperm are motile for six hours

## 3 Count of spermatozoa

This is accomplished by dilution and then using a counting chamber as in counting blood cells A 1 to 20 dilution is usually satisfactory and is prepared by measuring 0 5 cc in a pipette and adding 9 5 cc of diluting fluid (saturated sodium carbonate and 1% phenol) After mixing the sperm suspension is transferred to a counting chamber using a dropper pipette Five blocks of 16 squares are counted as in a red blood cell count and the number of spermatozoa per cc is calculated by adding 6 ciphers to the total number counted If the count is low the mixture may be shaken with 1 drop of HCl and the entire RBC field (25 squares—1 square mm) counted and 4 zeros added *Normally* 90 000 000 to 200 000 000 per cc are found Counts of 20 to 60 million indicate *relative infertility* although occasionally pregnancies do occur with counts of from 2 to 15 million per cc The sperm count

may vary considerably in patients with oligospermia and it is advisable to examine more than one specimen

4 Types of spermatozoa and incidence of pathological forms

For this portion of the examination a drop of semen is placed on a microscope slide and spread with another slide as in making a blood smear The smear is fixed with heat and then covered with 1% solution of chloramine for several minutes (This removes mucus and overlying spermatozoa so should not be used if very few are present ) The slide is then washed with water and 95% alcohol and dried by blotting with filter paper It is then stained for two to five minutes using Ziehl Neelsen s carbol fuchsin two parts concentrated alcoholic solution of eosin one part and 95% alcohol one part Following this it is washed with water and counterstained with Loeffler s methylene blue for a few seconds It is then washed dried and examined under the oil immersion lens The heads stain a purplish color while the tails and middle portions are red Usually 300 spermatozoa are counted noting the following characteristics for classification

1 *Normal spermatozoa*—usually over 70% of the total

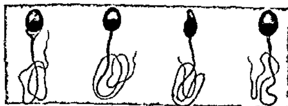


FIGURE 16 Normal spermatozoa showing head with full oval shape (After R S Hotchkiss *Infertility in Men* American Lecture Series No 53 Ed Willard O Thompson M D Springfield Ill Charles C Thomas Publisher 1952)

These present a head with full oval shape Some vari ations in size and shape may occur

2 *Abnormal nucleus*—the nucleus is at the base of the head of the sperm and should present a rounded *posterior angle Varying degrees of acuteness of this* angle indicate abnormality These are significant if over 10% of the number counted

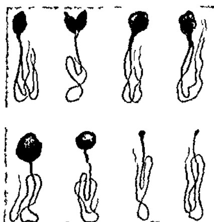


FIGURE 17 Abnormal spermatozoa showing duplicate forms (*above*) giant heads (megalospermia) and pin heads (microspermia) (*below*) (After R S Hotchkiss *Infertility in Men* American Lec ture Series No 53 Ed Willard O Thompson M D Springfield Ill Charles C Thomas Publisher 1952)

3 *Microspermia*—this is caused by deficient acrosome which forms the anterior portion of the head of the spermatozoa They are occasionally so small as to be classified as pin heads Normally they should not be more than 15% of the number counted

4 *Megalospermia*—usually not over 5% Many large forms may indicate pathologic spermatogenesis

5 *Abnormal acrosomia*—this refers to other abnormali ties of the head besides those noted in microspermia and should not exceed over 5% of the total count

6 *Miscellaneous forms*—these refer to abnormalities in staining cytoplasmic extrusions and abnormalities of the central part of the sperm or tail They should not exceed 5%

*Blom*[1] has suggested a *stain* which is occasionally of value in indicating *viability of sperm* even though they are not motile For this a small drop of semen about twice the size of a pinhead is placed on a clean slide Next a drop of 5% eosin (bluish) about double the size is placed beside it and finally a drop of 10% nigrosin double the size of the eosin drop With a glass rod the semen and eosin drops are mixed and then the nigrosin drop is included After a few seconds the mixture is spread as a thin layer with the glass rod held flat The mixing and smearing should not take over 1 minute The slide is then dried over a flame and examined under the oil immersion lens Dead cells will appear red having taken up the eosin stain while the live cells re main un colored For a differential enumeration 200 to 500 spermatozoa may be counted

Other material found in semen

*a* *White blood cells*—these are usually associated with infection in the urinary tract either in the prostate or the urethra

*b* *Tissue cells*—cells from the bladder urethra or other parts of the genito urinary tract are occasionally seen

*c* *Crystals*—several types are observed and in the pres ence of germinal hypoplasia they may be abundant

In any type of *hypogonadism* whether primary or second ary the volume and sperm concentration will be dimin ished As noted above in severe hypogonadism it may be impossible to obtain a specimen It is of interest to recall that in *Klinefelter s syndrome* there may be spermatogenic failure with normal Leydig-cell function (Other charac

teristics of the syndrome include gynecomastia and increased excretion of FSH in the urine) *Local pathology* within the testicle may be the cause of deficient sperm production and *represents a major portion of the problem* in the study of *infertility* in men

Hamblen Pullen and Cuyler[6] reported a study of the *correlation* of findings in seminal fluid with the level of *basal metabolic rates* done in males undergoing routine studies because of presumed sterility They found no consistent relationship indeed patients with basal metabolic rates below minus 15% generally produced better seminal specimens than those within the normal range

The estimation of *fructose concentration* in semen has recently been a subject of some interest as an *index of androgenic activity* Originally the estimation was found to be of value as an indicator of the fertility of bull semen Recently Landau and Loughead[7] have studied its concentration in man—noting that fructose apparently is secreted by the seminal vesicle and metabolized by spermatozoa They reported that in *normal men* the seminal fructose concentration ranged from 200 to 800 mg per 100 cc In *hypogonadism* the concentration was less than 135 mg per 100 cc and became elevated after the administration of androgens However patients with oligospermia or azoospermia who otherwise were normal had normal fructose concentration

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*Chapter XI*

# TESTIS BIOPSY

In the study of infertile men when the seminal fluid shows azoospermia or severe oligospermia or if no specimen of semen can be obtained a microscopic examination of the testicular tissue obtained by testis biopsy is indicated The simplicity of this procedure is surprising It was first performed experimentally by Engle and clinically by Hotchkiss as quoted by Charny [1] A study of the biopsy section obtained will give an absolute answer as to whether or not spermatozoa are being produced in proper numbers *in the seminiferous tubules of the testes The procedure* produces no deleterious effects on the human testis in contrast to damaging effects on testes of other mammalian species

Method This is well given by Hotchkiss After preparation of the genital region a small area of the scrotal skin is anesthetized with 1% novocaine A 2 to 3 cm incision is then made through the tunica vaginalis and a pair of ocular palpebral retractors inserted While holding the testicle in apposition to the wound a small V shaped *incision is made in the tunica albuginea and a piece of* tissue excised using small eye scissors Hemorrhage is arrested and the tunica and wound closed with silk or fine plain gut sutures This is repeated on the opposite side after which a suspensory is applied and the patient allowed to return to his home The tissue is fixed in Bouin s fluid Sections are prepared for microscopic examination as with other biopsy or pathological specimens



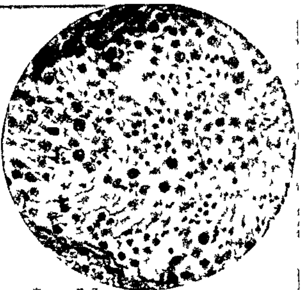


FIGURE 18 Normal testicular tissue (low and high power) Testis biopsy in a man with azoospermia caused by mechanical block Spermatozoa appeared in semen after vaso epididymal anastomosis and patient s wife became pregnant (Courtesy of Dr L Michelson—after *West J Surg Obst and Gynec* 55 120 Feb 1947 and *Surg Gynec and Obst* 82 327 March 1946)



FIGURE 19 Abnormal testicular tissue Testis biopsy showing tubular degeneration and absence of spermatogenesis Except for a few spermatogonia only Sertoli cells remain From a man aged 21 years with azoospermia but normal build and sexual function Had had severe electric shock at age of five years (Courtesy of Dr Lewis Michelson and Dr David A Wood)

*Examination of the specimen* will reveal the histologic basis for functional impairment In general 2 classes of tissue are found

1 *Normal testicular tissue* This shows normally functioning seminiferous tubules and adequate numbers of spermatozoa If the semen of such a patient does not show spermatozoa or presents severe oligospermia there is obviously a mechanical block between the testicle and the point of delivery into the urethra Minor histologic changes are occasionally seen which may be due to toxic nutritional or constitutional states which can be corrected
2 *Defective or abnormal tissue* Several variations are seen as follows

a Disorganized spermatogenesis with few mature sperm

b Germinal epithelium showing arrest of development at an early stage of spermatogenesis

c Tubules with little or no germinal epithelium



FIGURE 20 Abnormal testicular tissue Testis biopsy showing marked tubular degeneration following mumps Interstitial tissue is loosely cellular and Leydig cells are not prominent From a man aged 26 with azoospermia Had normal potency but some physical characteristics of hypogonadism (Courtesy of Dr Lewis Michelson and Dr David A Wood)

(germinal aplasia) and with no spermatozoa (see Figures 19 and 20)

d Proliferation of a large amount of fibrous tissue about the tubules

Changes in the interstitial or Leydig cells are also easily recognized in the sections

Engle[3] has suggested the following classification which correlates the semen analysis with testis biopsy

1 Normal semen
2 Azoospermia
  a with demonstrable occlusion
    1 normal tubules
    2 distorted spermatogenesis
  b without demonstrable occlusion
    1 normal tubules
    2 progressive tubular fibrosis
    3 germinal aplasia
    4 spermatogenic arrest (to spermatocyte I)
3 Oligospermia
  a with high frequency of abnormal spermatozoa
    1 incomplete maturation
    2 progressive tubular fibrosis
  b inflammation and infection

This has helped in evaluation of the fertility potential

**Clinical application** The biopsy may aid by indicating *proper therapy* e g if considerable amounts of *fibrosis and sclerosis* are seen it is apparent that the testicle has sustained severe direct damage from the etiologic factor—as seen following the orchitis of mumps or after x ray therapy The fault therefore is primary in the testicle which is obviously incapable of responding to stimulation therapy so substitution therapy with androgens is indicated

However if the tubules are *immature* and the *interstitial cells prepuberal* the lesion is probably secondary to lack of pituitary stimulation It is known from animal investigation that the pituitary gonadotropin which stimulates the follicles in the ovary (follicle stimulating hormone—FSH) is the one which in the male testis stimulates tubular development and function This would seem to indicate treatment with preparations of gonadotropins in these patients but unfortunately use of those now available has usually been disappointing in human subjects

*Should the testicular tissue appear normal* a search for the obstruction in the vas deferens is indicated with the possibility of plastic surgical relief (Figure 18)

If treatment is instituted with the intent of improving the testicle repeat testis biopsies can be performed at intervals in order to follow progress from therapy

*Another classification of testicular deficiency* has been suggested by Howard *et al*[4] which correlates the picture of testis biopsy with clinical and other laboratory findings From their interesting and thorough study they concluded that the following was satisfactory

*a* Patients with low FSH excretion
   1 cases without pituitary stimulation with prepuberal appearance of testes
   2 cases with decreased pituitary function after a period of normal function—testes characterized by hypospermatogenesis lipoid accumulation in the Sertoli cells thickening of the tubular tunica propria deposition of peritubular collagen and elastic tissue and absence of normal Leydig cells

*b* Patients with normal FSH excretion
   1 cases with endocrine disturbances—changes thought due to lack of luteinizing (interstitial cell stimulating) hormone — acromegaly
   2 cases with spermatogenic failure only and without other evidence of endocrine disease—these include arrest of maturation hypospermatogenesis and oligospermia

*c* Patients with high FSH excretion—this group includes patients with absent germ cells sclerosing tubular degeneration mumps orchitis male climacteric and absence of testes

Nelson and Heller[5] have also made careful studies including histochemical of the testicular lipides and have pointed out that men with primary hypogonadism with

few exceptions have high levels of excretion of urinary gonadotropins and therefore should not be treated with gonadotropic substances

The testis biopsy is occasionally of interest and may provide crucial evidence in making the diagnosis of the *Klinefelter syndrome* Sections of the testis show varying



FIGURE 21 Testis biopsy in Klinefelter s Syndrome Note tubular degeneration and prominence of interstitial tissue Patient aged 24 had gynecomastia normal sized genitalia and increased FSH excretion However sexual function was poor and improved with testosterone therapy (Courtesy of Dr Frank Hinman Jr and Dr Gilbert Gordan )

degrees of tubular lesions with partial to complete hyalinization and loss of spermatogenesis The interstitial or Leydig cells are numerous and prominent and are considered to indicate that the production of male hormone in these patients is normal However occasional cases do show evidence of some degree of hormonal deficiency

Recently Sobel Sniffen and Talbot[8] have reported that testicular biopsy may be of value in boys with *sexual precocity* in differentiating simple premature masculinity

from precocity caused by overactivity of the adrenal cortex In the patients with *premature masculinity* all testicular elements are stimulated—Leydig cells are seen and varying degrees of spermatogenesis are present In *precocity* of *adrenal cortical origin* Leydig cells are not activated and spermatogenesis is not seen However some tubular development does occur

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*Chapter XII*

# ENDOCRINE DISEASE INDEX

In clinical practice it is helpful to know what laboratory results may be expected in certain diseases and to be familiar with the tests which are of particular aid in establishing the diagnosis Therefore in this final chapter the material presented has been rearranged with the tests grouped under the headings of the various endocrinopathies The order of listing will be anatomical with the disease conditions assigned to the various glands primarily affected—starting with the pituitary Disorders caused by hyperfunction will be listed first followed by those ascribed to hypofunction A few conditions not clearly related to one gland or even not primarily endocrine but which show important findings in the laboratory tests discussed will be listed at the end

The laboratory tests will be divided into the following categories

1. Those most important in the disease which probably should be performed in every case
2. Those of interest in the disease which are occasionally helpful in diagnosis or in following the effects of treatment
3. Abnormal laboratory findings of incidental interest in the disease

The more important findings will be shown in heavier type

## PITUITARY DISEASES

### Acromegaly

**(1) MOST IMPORTANT TESTS**

**X ray of skull** usually shows enlarged deformed sella turcica ballooning of frontal sinuses enlargement of maxillary sinuses long mandible causing prognathism change in bite spacing of teeth thickening of calvarium and increased prominence of external occipital protuberance (Chapter VI p 53)

**Glucose tolerance test** may show diabetic tendency or actual diabetes mellitus (Chapter III p 15)

**Basal metabolic rate** moderately elevated in active phase moderately low in burned out phase (Chapter V p 43)

**Urinary 17 Ketosteroids** excretion elevated in active phase may be low in burned out phase (Chapter VII p 69)

**(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL**

**Serum phosphorus** may be increased with active phase (Chapter III p 30)

**X ray of hands** may show lateral tufting of terminal phalanges—occasionally an early diagnostic sign (also occurs in toes but change is less striking) (Chapter VI p 63)

**Dental X rays** may show spacing of teeth and change in bite (Chapter VI p 55)

**X ray of chest** (antero posterior and lateral) may show enlarged heart (splanchnomegaly) prominence of sternum and enlarged vertebrae with kyphosis (Chapter VI p 57)

**Urinalysis** may show glycosuria or occasionally evidence of diabetes insipidus such as low specific gravity and increased 24 hour volume (Chapter II p 7)

**(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST**

**Complete blood count** may show eosinophilia and relative lymphocytosis (Chapter II p 6)

**Serum sodium** occasionally elevated (Chapter III p 28)

**Serum chlorides** occasionally elevated (Chapter III p 9)

**Serum potassium** level occasionally depressed (Chapter III p 29)

### Gigantism

(physiopathological process similar to that in acromegaly except that disease starts during childhood)

**(1) MOST IMPORTANT TESTS**

**X ray of skull** usually shows enlarged or deformed sella turcica acro-

megalic changes also may occur after epiphyseal closure (Chapter VI p 53)

Glucose tolerance test may show diabetic tendency or diabetes (Chapter III p 15)

Basal metabolic rate moderately elevated with active phase moderately low in burned out phase (Chapter V p 43)

Urinary 17 ketosteroids excretion elevated in active phase may be low in burned out phase (Chapter VII p 69)

(?) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Serum phosphorus may be increased with active phase (Chapter III p 25)

Bone age may be slightly retarded especially with secondary eunuchoidism (Chapter VI p 53)

Urinalysis may show glycosuria or occasionally evidence of diabetes insipidus such as low specific gravity and increased 24 hour volume (Chapter II p )

Dental X rays may show spacing of teeth (Chapter VI p 55)

## Anterior Pituitary Insufficiency—Adult

(of varying degrees—findings typical of extreme insufficiency (Simmonds disease) will be indicated)

(1) MOST IMPORTANT TESTS

X ray of skull may show enlarged or deformed sella turcica if tumor is cause of the disease (Chapter VI p 53)

Basal metabolic rate low may be very low (i e minus 40 to 0%) in Simmonds disease (Chapter V p 43)

Urinary 17 Ketosteroids usually low (Chapter VII p 69)

Urinary gonadotropins (FSH) usually low—important in diagnosis of hypogonadotropic eunuchoidism (Chapter VII p 7 )

Glucose tolerance test low flat curve with tendency to hypoglycemia latter may be marked in Simmonds disease (Chapter III p 15)

Insulin tolerance test shows sensitivity to insulin may be marked to the point of danger in Simmonds disease—if this condition is suspected use ½ to ¼ recommended dose of insulin and have glucose ready for parenteral injection if necessary during course of test (Chapter III p 17)

(?) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Serum cholesterol normal or high in pituitary myxedema type—normal levels in a patient with myxedematous appearance should suggest

possibility of primary pituitary etiology (Chapter III p 17)
Eosinophile count normal or low (Chapter III p 10)
Corticotropin (ACTH) test eosinophiles usually drop more than 50%
(Chapter III p 11)
Serum sodium occasionally low with secondary diminution of adrenal cortical function (Chapter III p 28)
Serum chlorides occasionally low with secondary diminution of adrenal cortical function (Chapter III p 29)
Serum potassium occasionally high with secondary diminution of adrenal cortical function (Chapter III p 29)

**(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST**

Serum carotin occasionally increased causing carotinemia (Chapter III p 23)
Electrocardiogram shows bradycardia and small complexes changes more marked in Simmonds disease (Chapter V p 47)
Chest X ray may show small heart (splanchnomicria) especially in Simmonds disease (Chapter VI p 57)
Complete blood count may show secondary anemia and eosinophilia more frequent in Simmonds disease (Chapter II p 6)
Gastric analysis may show hypoacidity or achlorhydria especially in Simmonds disease (Chapter V p 46)
Urinalysis occasionally shows high 24 hour volume and low specific gravity with diabetes insipidus (Chapter II p 7)
Serum calcium may be moderately low (Chapter III p 24)
Endometrial biopsy shows ovarian failure with little proliferative and no secretory change most marked in severe insufficiency (Chapter IX p 93)
Semen examination volume small sperm absent or in low concentration fructose concentration low specimen may be unobtainable in severe hypopituitarism (Chapter X p 96)

## Hypophyseal Infantilism

(physiopathological process similar to that in adult anterior pituitary insufficiency except that disease starts in childhood)

**(1) MOST IMPORTANT TESTS**

X ray of skull may show enlarged or deformed sella turcica if tumor is cause of the disease (Chapter VI p 53)
Basal metabolic rate moderately low (Chapter V p 43)
Urinary 17 ketosteroids low excretion for age (Chapter VII p 69)
Urinary gonadotropins (FSH) low or absent (Chapter VII p 7 )
Bone age moderately retarded in childhood (Chapter VI p 53)

(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Glucose tolerance test low flat curve with tendency to hypoglycemia (Chapter III p 15)

Plasma cholesterol slightly low (Chapter III p 17)

(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST

Blood counts may show relative lymphocytosis (Chapter II p 6)

Urinalysis occasionally shows high 24 hour volume and low specific gravity if diabetes insipidus is present (Chapter II p 7)

Dental X rays may show crowding of teeth in small arch (Chapter VI p 55)

## Froehlich Syndrome

(diagnosis considered only in the classical sense when condition is caused by a pituitary tumor as in Froehlich s original case)

*(1) MOST IMPORTANT TESTS*

X ray of skull shows enlarged or deformed sella turcica (Chapter VI p 53)

Urinalysis occasionally shows high 24 hour volume and low specific gravity if diabetes insipidus is present (Chapter II p 7)

Other findings characteristic of hypopituitarism may be present

## Diabetes Insipidus

(1) MOST IMPORTANT TESTS

Urinalysis increased 24 hour volume (3 to 36 liters) low specific gravity (1 001 to 1 003) (Chapter II p 7)

X ray of skull sella turcica enlarged or deformed if tumor is causative factor (Chapter VI p 53)

(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Blood serology positive in occasional case caused by central nervous system syphilis (Chapter II p 9)

Urine concentration test usually does not raise urine concentration to above 1 005 (Chapter IV p 37)

Carter Robbins urinary excretion test helps to differentiate the disease from psychogenic polydipsia (Chapter IV p 36)

Glucose tolerance test normal curve distinguishes the disease from diabetes mellitus (Chapter III p 15)

## THYROID DISEASES

### Hyperthyroidism

(includes that resulting from hyperplasia or toxic adenoma and exophthalmic goiter)

**(1) MOST IMPORTANT TESTS**

**Basal metabolic rate** level increased (Chapter V p 43)
**Blood protein bound iodine** level increased (Chapter III p 21)
**Plasma cholesterol** low or low normal (Chapter III p 17)

**(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL**

**Uptake of radioactive iodine** high curve with rapid rise to over 30% in five hours (Chapter V p 45)
**Glucose tolerance test** may show sharp rise to high level and then rapid fall (Chapter III p 15)
**Urinalysis** may show glycosuria (Chapter II p 7)
**Electrocardiogram** shows rapid rate with tall R and T waves—occasionally auricular fibrillation or flutter (which may be paroxysmal)—late in disease signs of left ventricular hypertrophy may be present (Chapter V p 47)
**Chest X ray** may show evidence of substernal goiter or deviation of the trachea—late in disease may show cardiac hypertrophy or dilatation (Chapter VI p 57)

**(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST**

**Blood counts** may show leucopenia with relative lymphocytosis when disease is severe eosinophilia and secondary anemia may be present (Chapter II p 6)
**Serum sodium** occasionally low (Chapter III p "8)
**Creatine in urine** occasionally present in adult men (Chapter IV p 40)

### Hypothyroidism

(of varying degrees—in adults and children changes characteristic of extreme insufficiency (myxedema) will be indicated)

**(1) MOST IMPORTANT TESTS**

**Basal metabolic rate** level decreased (Chapter V p 43)
**Blood protein bound iodine** low or low normal (Chapter III p 21)
**Plasma cholesterol** level increased (if normal level is found consider diagnosis of pituitary myxedema) (Chapter III p 17)
**Chest X ray** may reveal large flabby atonic myxedema heart or show evidence of pericardial fluid or pleural fluid (Chapter VI p 57)

**Blood counts** secondary anemia frequent primary anemia occasionally seen relative lymphocytosis (Chapter II p 6)

**Urinalysis** albumin and signs of chronic infection resulting from bladder atony and residual urine (Chapter II p 7)

**Bone age** (important in childhood) shows marked retardation in childhood myxedema (Chapter VI p 53)

### (2) TESTS OF INTEREST—OCCASIONALLY HELPFUL

**Uptake of radioactive iodine** low uptake curve (Chapter V p 45)

**Serum carotin** usually increased so that carotinemia is present in untreated myxedema (Chapter III p 93)

**Glucose tolerance test** curve tends to be low and flat (Chapter III p 15)

**Electrocardiogram** shows bradycardia prolonged P R interval low voltage changes in S T segments and depressed or inverted T waves—changes most marked in definite myxedema and are reversible with treatment (Chapter V p 47)

**Urinary 17 Ketosteroids** excretion usually low (Chapter VII p 69)

**Dental X rays** may show retarded dentition in children (Chapter VI p 55)

**Gastrointestinal x rays** demonstrate atony and sluggish function of bowel (Chapter VI p 9)

**Pyelograms—(intravenous or retrograde)** may demonstrate atony of bladder and residual urine (Chapter VI p 59)

### (3) LABORATORY FINDINGS OF INCIDENTAL INTEREST

**Creatine in urine** may be absent in childhood type of myxedema (Chapter IV p 40)

**Gastric analysis** acidity usually low with occasional achlorhydria (Chapter V p 46)

**Serum sodium** occasionally slightly high (Chapter III p 98)

**Endometrial biopsy** may show excessive bleeding from proliferative endometrium—also occasionally from secretory endometrium (Chapter IX p 91)

## PARATHYROID DISEASES

### Hyperparathyroidism

#### (1) MOST IMPORTANT TESTS

**Serum calcium** level increased (Chapter III p 24)

**Serum phosphorus** level depressed (Chapter III p 2 )

**Serum alkaline phosphatase** level increased with bone involvement—*low if no bone involvement* (Chapter III p 26)

**Urine Sulkowitch** strongly positive (Chapter IV p 38)

Urinalysis calcium casts may be found late in disease renal stones usually present causing albumin red blood cells white blood cells and casts (Chapter II p 7)

X ray bone studies may show osteoporosis or the typical widely distributed subperiosteal cysts (osteitis fibrosa cystica of von Recklinghausen) (Chapter VI pp 64-65)

## ( ) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Pyelograms (intravenous or retrograde) in late cases may demonstrate nephrocalcinosis renal lithiasis or pyelonephritis (Chapter VI p 59)

Dental X rays show disappearance of lamina dura around roots of teeth (Chapter VI p 55)

Blood non protein nitrogen may be elevated in terminal cases (Chapter VII p 39)

## (3) LABORATORY FINDINGS OF INCIDENTAL INTEREST

Blood counts occasionally show secondary anemia and eosinophilia (Chapter II p 6)

Chest X ray cardiac enlargement may be found in late cases (Chapter VI p 54)

# Hypoparathyroidism
## (parathyroid tetany)

## (1) MOST IMPORTANT TESTS

Serum calcium level depressed (Chapter III p 24)
Serum phosphorus level increased (Chapter III p 25)
Urine Sulkowitch results negative or weakly positive (Chapter IV p 38)

## ( ) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Ellsworth Howard test shows increase in urinary phosphorus (important in differentiation from pseudohypoparathyroidism) (Chapter IV p 40)

*Dental X rays may show enamel defects and blunting of dental roots* (Chapter VI p 55)

## (3) LABORATORY FINDINGS OF INCIDENTAL INTEREST

Plasma CO combining power occasionally slightly increased (Chapter III p 3 )

Basal metabolic rate occasionally moderately elevated (Chapter V p 43)

Blood counts may show polycythemia and relative lymphocytosis (Chapter II p 6)

## Pseudohypoparathyroidism

(Seabright Bantam syndrome)

(1) MOST IMPORTANT TEST

Ellsworth Howard test shows constant excretion of phosphorus (Chapter IV p 40)

# DISEASES OF PANCREATIC ISLETS

## Hyperinsulinism

(1) MOST IMPORTANT TEST

Glucose tolerance test shows low fasting level and low curve with tendency to hypoglycemic dip—tests should be run from 5 to 6 hours and extra blood specimen taken if hypoglycemic symptoms occur (Chapter III p 15)

(2) TEST OF INTEREST—OCCASIONALLY HELPFUL

Insulin tolerance test shows sensitivity to insulin with low blood sugar levels—may induce hypoglycemic attack and parenteral glucose should be ready for injection if necessary (Chapter III p 17)

(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST

Plasma cholesterol may be slightly low (Chapter III p 17)
Serum phosphorus may be slightly low (Chapter III p 25)

## Diabetes Mellitus

(including acidosis)

(1) MOST IMPORTANT TESTS

Urinalysis shows sugar and high specific gravity in acidosis acetone and diacetic acid are present with Kimmelstiel Wilson syndrome albumin and casts are found (Chapter II p 7)
Glucose tolerance test shows high curve with slow fall (Chapter III p 15)
Plasma CO combining power low in acidosis (Chapter III p 39)

(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Plasma cholesterol tends to be high especially in acidosis (Chapter III p 17)
Blood non protein nitrogen may be elevated in acidosis (Chapter III p 32)
Electrocardiogram may show signs of low serum potassium as a complication of acidosis (Chapter V p 47)
Berger test 100 mg corticotropin (ACTH) before glucose tolerance test may indicate potential diabetes mellitus (Chapter III p 16)

**Serum phosphorus** may be slightly high and does not fall normally during intravenous glucose tolerance test (Chapter III p 25)

**(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST**

**Urine creatine** occasionally present in adult men (Chapter IV p 40)

**Urinary 17 Ketosteroids** excretion may be slightly low (Chapter VII p 69)

**Blood counts** may show polycythemia and leucocytosis in acidosis (Chapter II p 6)

## DISEASES OF THE ADRENALS

### Cushing s Syndrome

### (hyperadrenocorticism)

**(1) MOST IMPORTANT TESTS**

**Glucose tolerance test** shows tendency to diabetic type of curve (Chapter III p 15)

**Urinalysis** may show glycosuria in late hypertensive phase may show albumin casts and fixed specific gravity (Chapter II p 7)

**Blood counts** show characteristic polycythemia (Chapter II p 6)

**Urinary excretion of 11-oxysteroids or glycogenic corticoids or Compound E & F steroids** usually increased (Chapter VII p 66)

**X rays of bones** may show osteoporosis including collapse of vertebrae (Chapter VI p 64)

**( ) TESTS OF INTEREST—OCCASIONALLY HELPFUL**

**Urinary 17 Ketosteroids** may be slightly elevated higher if condition is result of adrenal cortical tumor (Chapter VII p 69)

**Abdominal X rays including pyelograms (either intravenous or retrograde)** Causative adrenal tumor may cast soft tissue shadow or cause downward displacement of kidney or deformity of upper calyx (Chapter VI p 59)

**Extraperitoneal pneumography (retroperitoneal gas)** may demonstrate causative tumor by contrast (Chapter VI p 61)

**Serum sodium** may be elevated (Chapter III p 28)

**Serum chloride** may be elevated (Chapter III p 23)

**Serum potassium** may be lowered (Chapter III p 29)

**X ray of chest** may show enlargement of the heart late in course of disease with lateral view demonstrating possible collapse of vertebrae from osteoporosis (Chapter VI p 57)

**Electrocardiogram** may show signs of left ventricular hypertrophy and myocardial damage late in course of disease (Chapter V p 47)

**Blood non protein nitrogen** may be elevated in terminal phase of disease (Chapter III p 3 )

(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST

Basal metabolic rate may be moderately elevated (Chapter V p 43)
Plasma cholesterol may be slightly high (Chapter III p 17)

## Adrenal Cortical Tumor

(occasional patients with Cushing s syndrome are in this category—childhood syndromes (adrenogenital) include precocious puberty in boys precocious puberty in the masculine direction in girls and very rarely gynecomastia in boys)

(1) MOST IMPORTANT TESTS

Urinary 17 Ketosteroids excretion usually increased reaching levels of 50 to over 1 000 mg per 24 hours rarely however the excretion may be normal if beta fraction is over 30% with a total excretion of over 50 mg carcinoma is likely (Chapter VII p 69)

Abdominal X rays including pyelograms (either intravenous or retrograde) tumor may cast soft tissue shadow or cause downward displacement of kidney or deformity of the upper calyx (Chapter VI p 59)

Extraperitoneal pneumography (retroperitoneal gas) demonstrates tumor by contrast (Chapter VI p 61)

Glucose tolerance test may show diabetic type of curve (Chapter III p 15)

Urinalysis may show glycosuria (Chapter II p 7)

Allen test (for dehydroisoandrosterone in urine) positive with tumor—may be helpful in differentiation from adrenal cortical hyperplasia (Chapter VII p 67)

Bone age advanced with pubertus praecox in childhood (Chapter VI p 53)

(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Serum sodium may be elevated (Chapter III p 28)
Serum chloride may be elevated (Chapter III p 29)
Serum potassium may be low (Chapter III p 29)

Perirenal air (or gas) insufflation may help to demonstrate the tumor by contrast a relatively dangerous procedure (Chapter VI p 59)

Arteriography may demonstrate circulation to the tumor (Chapter VI p 65)

Testis biopsy in boys with sexual precocity—shows some tubular development but no spermatogenesis and no activation of Leydig cells may help to differentiate from other types of precocious puberty (Chapter XI p 110)

Dental X rays may show advanced dentition with precocious puberty (Chapter VI p 53)

(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST

Endometrial biopsy may show atrophy of endometrium (similar to picture seen following large doses of androgens) (Chapter IX p 91)
Basal metabolic rate may be moderately elevated (Chapter V p 45)
Plasma cholesterol may be slightly high (Chapter III p 14)

## Adrenal Cortical Hyperplasia

(Many laboratory findings are similar to those found in adrenal cortical tumor though changes are usually less marked)

(1) MOST IMPORTANT TESTS

Urinary 17 Ketosteroids excretion usually increased usual range 30 to 100 mg per 24 hours (Chapter VII p 69)
X ray studies of adrenal regions (including pyelograms and contrast studies) no evidence of tumor seen (Chapter VI pp 59 & 61)
Allen test (for dehydroisoandrosterone in the urine) negative—may be helpful in differentiation from adrenal cortical tumor (Chapter VII p 67)
Glucose tolerance test may show diabetic type of curve (Chapter III p 15)
Urinalysis may show glycosuria (Chapter II p 7)
Bone age advanced with pubertas praecox in childhood (Chapter VI p 53)

( ) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Testis biopsy in boys with sexual precocity—shows some tubular development but no permatogenesis and no activation of Leydig cells may help to differentiate from other types of precocious puberty (Chapter XI p 110)
Dental X rays may show advanced dentition with precocious puberty (Chapter VI p )

(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST

Endometrial biopsy may show atrophy of endometrium (similar to picture seen following large doses of androgens) (Chapter IX p 91)

## Adrenal Medullary Tumor

(Pheochromocytoma)

(1) MOST IMPORTANT TESTS

Benzodioxane test usually causes drop in blood pressure during periods of hypertension (Chapter V p 50)
Regitine test usually causes marked fall in blood pressure during periods of hypertension (Chapter V p 0)

Abdominal X rays including pyelograms (either intravenous or retrograde) tumor may cast soft tissue shadow or cause downward displacement of kidney or deformity of the upper calyx (Chapter VI p 59)

Extraperitoneal pneumography (retroperitoneal gas) demonstrates tumor by contrast (Chapter VI p 61)

Caution—pressure from gas on tumor may precipitate attack of paroxysmal hypertension

(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Dibenamine test causes decrease in blood pressure if hypertension is present (Chapter V p 51)

Histamine test may cause marked increase in blood pressure and provoke a characteristic attack (increase in blood pressure may be dangerous) (Chapter V p 51)

Mecholyl test (acetyl beta methyl-choline) may induce hypertensive attack (may be dangerous) (Chapter V p 51)

Tetraethylammonium test usually induces a paroxysmal hypertensive attack (may be dangerous) (Chapter V p 1)

Cold pressor test not a likely to produce hypertension a differential point from other types of labile hypertension (may be dangerous) (Chapter V p 51)

(3) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Basal metabolic rate may be elevated (Chapter V p 43)

Glucose tolerance test may show diabetic type of curve (Chapter III p 15)

Urinalysis may show sugar and albumin especially during attacks (Chapter II p 7)

Electrocardiogram may show tachycardia and extrasystoles during attacks (Chapter V p 47)

## Addison s Disease

(1) MOST IMPORTANT TESTS

Serum sodium level depressed (Chapter III p 28)

Serum chloride level depressed (Chapter III p 29)

Serum potassium level increased (Chapter III p 29)

Robinson Power Kepler water-excretion test demonstrates slow excretion of water and A factor bel w (Chapter IV p 35)

Circulating eosinophile count high or high normal (Chapter III p 10)

Corticotropin (ACTH) test (4 hours and 48 hours) shows failure of the circulating eosinophiles to drop 0% or more (Chapter III p 11)

Urinary 17 Ketosteroids excretion low may approximate 0 in women (Chapter VII p 69)

**Glucose tolerance test** shows low flat curve with tendency to hypoglycemia (Chapter III p 15)

**Insulin tolerance test** may demonstrate extreme sensitivity to insulin—if this condition is suspected use ½ to ¼ recommended dose of insulin and have glucose ready for parenteral injection if necessary during test (Chapter III p 17)

**Blood non protein nitrogen** may be elevated in crisis (Chapter III p 39)

**(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL**

**Cutler Power Wilder test** shows increased excretion of urinary chlorides (hospitalization necessary—may precipitate crisis) (Chapter IV p 34)

**Chest X ray** may show tuberculosis (Chapter VI p 57)

**Abdominal X rays** may show calcification of adrenal glands (Chapter VI p 59)

**Blood counts** may show secondary anemia eosinophilia and relative lymphocytosis (Chapter II p 6)

**Gastric analysis** frequently shows low gastric acidity or achlorhydria (Chapter V p 46)

**(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST**

**Basal metabolic rate** may be moderately low (Chapter V p 43)

**Plasma cholesterol** may be slightly low (Chapter III p 17)

**Serum calcium** may be moderately low (Chapter III p 21)

**Serum phosphorus** may be slightly high in crisis (Chapter III p 25)

**Plasma CO combining power** occasionally low (Chapter III p 39)

## Simple Hirsutism

**(1) MOST IMPORTANT TEST**

**Urinary 17 Ketosteroid** may be slightly increased—to levels of 25 to 30 mg per 24 hours Higher levels (70 mg or above) suggest adrenal cortical tumor (Chapter VII p 69)

# GONADS—DISEASES AND ASSOCIATED CONDITIONS

## Pregnancy

**(1) MOST IMPORTANT TESTS**

**Hormone excretion tests**

(Ascheim Zondek test Freidman test South American male toad test (Galli Mainini) North American male frog test South African female clawed frog test and Guterman test) Positive (Chapter VII p 67)

**(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL**

**Vaginal smear** shows high percentage of cornification at first but after two to four weeks increased number of folded basophilic and mid

zonal cells are seen with *threatened or incomplete abortion* red cells appear as well as leucocytes and histiocytes often in clusters or rosettes (Chapter VIII p 86)

Urinary 17 Ketosteroids excretion slightly increased (Chapter VII p 69)

Pregnandiol excretion elevated level falls with threatened abortion (Chapter VII p 78)

## Endocrine Ovarian Tumors

### (2) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Urinary 17 Ketosteroids may be slightly increased in *hyperthecosis* and in *arrhenoblastoma* (Chapter VII p 69)

Endometrial biopsy with *granulosa theca cell* tumors may show evidences of hyperestrinism such as cystic hyperplasia or decidua like changes—occasionally secretory changes are seen with *arrhenoblastoma* atrophic endometrium may be found (Chapter IX pp 93 & 94)

Urinary pregnandiol excretion may be increased with *polycystic ovaries* (Stein Leventhal syndrome) (Chapter VII p 78)

Cervical mucus profuse clear and thin with *polycystic ovaries* (Chapter VIII p 88)

### (3) LABORATORY FINDINGS OF INCIDENTAL INTEREST

Basal metabolic rate may be moderately elevated in *arrhenoblastoma* (Chapter V p 43)

Glucose tolerance test occasionally shows diabetic type of curve in *arrhenoblastoma* (Chapter III p 1 )

Serum sodium and chloride may be slightly elevated in *arrhenoblastoma* (Chapter III pp 28 & 29)

Serum postassium may be slightly low in *arrhenoblastoma* (Chapter III p 29)

## Menopause Syndrome

### (1) MOST IMPORTANT TEST

Vaginal smear shows varying degrees of estrogen deficiency—if *mild* most cells from intermediate layer and cornified cells 20% or under if *moderate* most cells from basal layer some intermediate cells seen and percentage of cornification low if *severe* nearly all cells from basal layer leucocytes and bacteria prominent (Chapter VIII p 85)

### (2) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Urinary gonadotropins (FSH) usually elevated (Chapter VII p 75)

Urinary 17 Ketosteroids slightly diminished level further depressed in old age (Chapter VII p 69)

Endometrial biopsy shows little or scant proliferative change and no secretory change (Chapter IX p 37)

**X rays of bones** occasionally show osteoporosis more frequent in later years (Chapter VI p 64)

## Ovarian Aplasia

### (Ovarian agenesis Turner s syndrome)

**(1) MOST IMPORTANT TEST**

**Urinary gonadotropins (FSH)** high—(patient should not have received estrogens for one month before test is performed) (Chapter VII p 75)

**(2) TEST OF INTEREST—OCCASIONALLY HELPFUL**

**X rays of bones** may show osteoporosis or evidences of congenital deformities (Chapter VI p 64)

**Bone age** only slightly retarded (Chapter VI p 53)

## Cancer of the Lower Female Genital Tract

**(1) IMPORTANT TEST**

**Vaginal smear (Papanicolaou stain)** 96% accuracy with squamous-cell carcinoma 80% accuracy with endometrial or fundal carcinoma (Chapter VIII p 87)

**(2) TEST OF INTEREST—OCCASIONALLY HELPFUL**

**Endometrial biopsy** shows carcinoma in 75% of cases—D & C is more certain and is therefore preferable (Chapter IX p 94)

## Tuberculosis of Endometrium

**(1) IMPORTANT TEST**

**Endometrial biopsy** occasionally shows tuberculous histopathology (Chapter IX p 94)

## Hypogonadism

### (male and female—includes eunuchoidism)

**(1) MOST IMPORTANT TESTS**

**Urinary 17-ketosteroids** excretion diminished in males but may be slightly elevated (Chapter VII p 69)

**Urinary gonadotropins (FSH)** excretion increased in eunuchs female castrates and in some eunuchoids in latter may also be normal or low (Chapter VII p 75)

**(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL**

**Bone age** moderately retarded with delayed epiphyseal closure in childhood and early adult life (Chapter VI p 53)

*Basal metabolic rate* may be moderately low (Chapter V p 43)

Urinary creatine may be present in adult males (Chapter IV p 40)
Serum carotin occasionally increased causing carotinemia (Chapter III p 23)
Vaginal smear shows nearly all basal cells with low percentage of cornification leucocytes and bacteria prominent (similar picture seen following androgen therapy) (Chapter VIII p 87) *Iodine vapor stain* shows low glycogen index (Chapter VIII p 89)
Endometrial biopsy shows little or no proliferative change and no secretory change serial studies every one to two weeks will show any evidence of cycle or ovulation (Chapter IX p 92)
Semen examination volume is diminished and sperm concentration low or absent fructose concentration low (specimen may be unobtainable) (Chapter X p 96)

(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST

Serum calcium may be moderately low (Chapter III p 21)
Blood count frequently shows a relative lymphocytosis (Chapter II p 6)

## Infertility
### (male and female)

(1) MOST IMPORTANT TESTS

Semen examination *volume* decreased with hypogonadism abnormal *transparency* and watery *viscosity* associated with low sperm concentration (Chapter X p 97)
*sperm count* shows oligospermia (under 0 million per cc) or with relative infertility 20 to 60 million per cc or azoospermia—the latter may be from local pathology in testicle (Chapter X p 98)
*impaired motility* of sperm may be seen (Chapter X p 98)
*abnormal forms* of sperm over 30% in differential count (Chapter X p 99)
high percentage of non viable cells by *Blom stain* (Chapter X p 101)
abundant *crystals* with germinal hypoplasia (Chapter X p 101)
Endometrial biopsy may show *estrogen deficiency* with little or no change (Chapter IX p 92) or progesterone deficiency (evidence of anovulatory cycle) with no secretory change (Chapter IX p 92)

(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL

Testis biopsy may be *normal* if mechanical block is present in vas or may show the following *abnormalities* disorganized spermatogenesis arrested development of germinal epithelium germinal aplasia tubular fibrosis and sclerosis or evidence of inflammation and infection (Chapter XI p 104)
Examination of cervical mucus may be *thick* (as opposed to thin at time of greatest fertility) (Chapter VIII p 88)

## Male Climacteric

**(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL**

Urinary gonadotropins (FSH) may be elevated (Chapter VII p 75)
Urinary 17 ketosteroids level slightly diminished may be further depressed in old age (Chapter VII p 69)

## Cancer of the Male Genital Organs

**(1) MOST IMPORTANT TESTS**

Serum acid phosphatase may be elevated in *carcinoma of the prostate* particularly if there is metastatic involvement of bone (Chapter III p 28)
Chorionepithelioma of testis gives *positive pregnancy test* (Ascheim Zondek Friedman male frog male toad etc) (Chapter VII p 69)

## Precocious Puberty

## (of pineal hypothalamic thymus or idiopathic origin)

**(1) MOST IMPORTANT TESTS**

Urinary 17 ketosteroids may be increased if clinical picture results from overstimulation of the adrenal cortex (Chapter VII p 69)
Bone age advanced over chronological age (Chapter VI p 53)
Testis biopsy in *simple premature masculinity* shows stimulation of all elements—Leydig cells are seen as well as varying degrees of spermatogenesis (Chapter XI p 110)
X ray of skull may show evidence of pineal or hypophalamic tumor (Chapter VI p 53)
Chest X ray may show evidence of thymic tumor (Chapter VI p 57)

**(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL**

Urinalysis may show increased 24 hour volume and low specific gravity if *diabetes insipidus* is present (Chapter II p 7)
Dental X ray may show advanced dentition (Chapter VI p 55)

**(3) LABORATORY FINDINGS OF INCIDENTAL INTEREST**

Basal metabolic rate may be moderately elevated (Chapter V p 43)
Serum sodium and chloride may be slightly high (Chapter III pp 28 & 29)
Serum potassium may be slightly low (Chapter III p 29)

## MISCELLANEOUS SYNDROMES—NOT NECESSARILY ENDOCRINE

## Klinefelter Syndrome

**(1) MOST IMPORTANT TESTS**

Urinary gonadotropins (FSH) excretion elevated (Chapter VII p 75)

Semen examination shows evidence of spermatogenic failure with oligospermia or azoospermia (Chapter X p 101)

**(2) TESTS OF INTEREST—OCCASIONALLY HELPFUL**

Testis biopsy shows partial to complete hyalinization of tubules interstitial (Leydig) cells numerous and prominent (Chapter XI p 110)

## Albright's Syndrome
### (polyostotic fibrous dysplasia)

**(1) IMPORTANT TEST**

X ray of bones may show characteristic disseminated osteitis fibrosa cystica in segmental distribution (Chapter VI p 6[illegible])

**(2) TEST OF INTEREST**

Serum alkaline phosphatase may be moderately elevated (Chapter III p 26)

## Osteoporosis

**(1) MOST IMPORTANT TESTS**

Urine Sulkowitch test strongly positive when condition is severe (Chapter IV p 38)
Serum calcium phosphorus and alkaline phosphatase normal (Chapter III pp 24 25 & 26)
Dental X rays may emphasize presence of osteoporosis by contrast (Chapter VI p 55)

## Paget's Disease of Bone

**(1) MOST IMPORTANT TESTS**

Urine Sulkowitch strongly positive (Chapter IV p 38)
Serum alkaline phosphatase high (Chapter III p 26)

**(2) TEST OF INTEREST**

Serum calcium occasionally slightly high (Chapter III p 24)

## Vitamin D Intoxication

**(1) MOST IMPORTANT TESTS**

Urine Sulkowitch test strongly positive (Chapter IV p 38)
Serum calcium elevated (Chapter III p 24)

## Anorexia Nervosa

(may be difficult to distinguish from Simmonds disease)

### (1) MOST IMPORTANT TESTS

Basal metabolic rate may be very low (Chapter V p 43)
Urinary 17 Ketosteroids normal or low (Chapter VII p 69)
Urinary gonadotropins (FSH) normal or low (Chapter VII p 75)

*This Book*

LABORATORY AIDS

IN

ENDOCRINE DIAGNOSIS

By Roberto F Escamilla M D

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